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Long-term alterations in the corticotropin-releasing hormone system: effects on emotional function and attention

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For Carla & Kim

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Abstract

Depression is one of the most common medical conditions worldwide. The most frequently observed neuroendocrine symptom among depressives is an enhanced secretion of the stress hormone cortisol. It has been hypothesised that corticotropin-releasing hormone (CRH) hypersecretion is, at least in part, responsible for these elevated cortisol levels. In addition, increased CRH levels in the CSF indicate that the hyperactivity of the CRH system is not limited to an augmented release of CRH into the portal vein, which indirectly enhances cortisol release, but possibly also in the brain. Therefore, it is possible that some of the symptoms, such as increased anxiety, altered endocrinological responses to 5-HT_{1A} receptor challenge and reduced attentional performance observed in depression are due to a hyperactive central CRH system. In this work, the role of CRH on these phenomena was investigated in mice.

In order to assess anxiety-related behaviour, a range of anxiety tests, based on different principles, were employed. Investigation of differences between four mouse strains and effects of long-term treatment with the selective serotonin reuptake inhibitor citalopram on anxiety-related behaviour revealed that the outcome of one test does not necessarily predict the outcome of another test based on different principles, suggesting that different aspects of anxiety can be taxed. Along similar lines, life-long overproduction of CRH increases anxiety-related behaviour in some paradigms, but not in all. Therefore, one might speculate that chronic CRH excess may increase some, but not all, aspects of anxiety. Treatment of CRH overexpressors revealed that chronic treatment with citalopram increased anxiety-related behaviour in wildtype mice, but induced opposite effects in CRH overexpressing mice, i.e., the

effects of antidepressants depended on baseline performance. Citalopram has beneficial effects in both patients suffering from anxiety disorders as well as depressive patients. These findings open the possibility that the beneficial effects of citalopram in depressed patients may at least in part depend on the activity of the CRH system seen in these patients, and that the elevation of CRH activity may be the cause of the occurrence of increased anxiety in this illness.

Furthermore, the results obtained with citalopram suggest important interactions between CRH and serotonin (5-HT). Related to this, a lack of increased HPA-axis activation after a 5-HT_{1A} receptor challenge was demonstrated, a phenomenon also reported in depressed patients. This contrasts an unaltered hypothermic response after such pharmacological challenge in CRH transgenic mice, which has also been reported in depressed patients.

Finally, the role of CRH in attentional processes, which are also impaired in depressed patients, were investigated. Lack of CRH did not affect visuospatial attention. In addition, lack of CRH receptor 2 or acute blockade of CRH receptor 1 did not affect attentional processes. Life-long loss of CRH receptor 1 resulted in a mild impairment. However, closer examination of performance of these animals suggest that this may not be a pure attentional deficit and opens the possibility that other factors contributed to these effects. In contrast, overproduction of CRH induced a more clear-cut impairment in visuospatial attention. Moreover, altered responsiveness to cholinergic drugs on visuospatial attentional performance observed in these mice point towards an interaction of the CRH system and the cholinergic neurotransmitter system in the modulation of these processes.

Taken together, overproduction of CRH increases some aspects of anxiety-related behaviour, blocks 5-HT_{1A} receptor mediated HPA-axis activation, and disrupts visuospatial attention, all symptoms that occur in depression. Therefore, inhibiting CRH activity may be a potential target for treatment of depression.

Chapter 1

Introduction

Discovery of CRH

Regulation of the pituitary-adrenocortical axis by the hypothalamus was first proposed by Sir Geoffrey Harris in 1948. This was followed by the discovery in 1955 that the presence of a factor in hypothalamic extracts could stimulate the release of adrenocorticotrophic hormone (ACTH; see p. 257 for a list of abbreviations used throughout the text) from pituitary cells in vivo (Guillemin and Rosenberg, 1955; Saffran and Schally, 1955). This factor was called corticotropin-releasing factor, later also known as corticotropin-releasing hormone (CRH). It was not until 1981 that CRH was purified and its chemical structure was discovered (Vale et al., 1981). Vale and colleagues showed that this 41-residue peptide is highly active in stimulating the secretion of ACTH and β -endorphin in vitro as well as in vivo. Stimulation of ACTH release in vivo occurs through release of CRH from neurons located in the hypothalamic paraventricular nucleus (PVN) into the portal vein. However, not only CRH can stimulate ACTH release, but also other molecules, such as arginine vasopressin (AVP), catecholamines and angiotensin II have these properties, albeit with weaker potency compared to CRH (De Wied et al., 1969; Vale et al., 1981). These molecules show synergistic effects with CRH on ACTH secretion

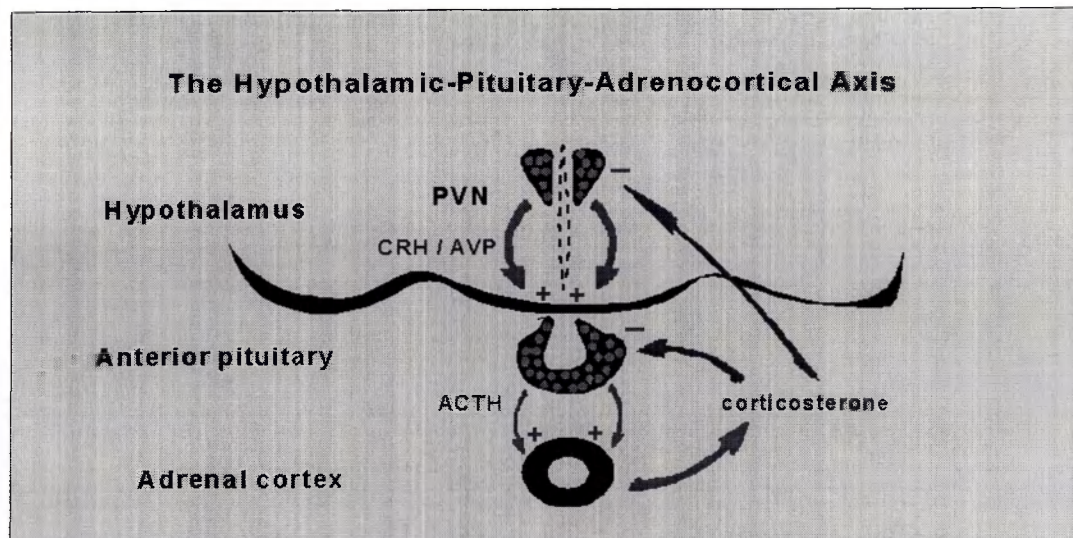


Figure 1. 1 Overview of the HPA-axis.

Thus, CRH seems to be the main activator of the hypothalamus-pituitary-adrenocortical (HPA) axis (Figure 1.1). ACTH in turn stimulates the adrenal cortex to release glucocorticoid hormones (cortisol in humans, and corticosterone in animals). The release of glucocorticoids shows circadian rhythmicity, which is under influence of the suprachiasmatic nuclei (Moore and Eichler, 1972; Watanabe and Hiroshige, 1981). Glucocorticoids are released in the peripheral circulation, where most of the glucocorticoids bind to corticosteroid-binding globulin. Only the “free” glucocorticoids are biologically active. Glucocorticoids bind to intracellular receptors. Two subtypes have been identified, mineralocorticoid receptors (MR) and glucocorticoid receptors (GR), which have been shown to differ in topography and occupation in the rat brain (Reul and de Kloet, 1985). These authors have demonstrated that MR are predominantly expressed in the hippocampus but also, for example, in the lateral septum, whereas the distribution of GR is more widespread in the brain. The highest density of GR is found in the lateral septum, dentate gyrus, the nucleus tractus solitarii and central amygdala, while moderate GR density can be

observed in the PVN and locus coeruleus. Lowest GR densities were found in the raphé nuclei, subiculum and hippocampal CA₁ field. MR have high affinity for corticosterone, which contrasts the affinity of the GR for corticosterone, which is about 6 to 10-fold lower. Therefore, the MR is extensively occupied with endogenous corticosterone under most circumstances. The GR becomes occupied after increasing plasma corticosterone levels, such as at the diurnal peak or after stress (Reul and de Kloet, 1985). From that, Reul and De Kloet suggested that the MR may be involved in tonic influence of brain function, whereas the GR may be involved in feedback action on stress-activated brain mechanisms.

Glucocorticoids inhibit the HPA-axis at several levels. One mechanism acts directly at the level of the parvocellular PVN and anterior pituitary (Swanson and Simmons, 1989), whereas indirect inhibition of HPA-axis activity occurs at the level of the ventral hippocampus, which projects to the bed nucleus of the stria terminalis (BNST). The latter inhibits HPA-axis activity via projections to the PVN (Cullinan et al., 1993). Furthermore, it has been shown that lesioning the medial prefrontal cortex results in an augmented ACTH and corticosterone release after restrained stress, but not after exposure to ether. This suggests that the medial prefrontal cortex plays a role in negative feedback effects on HPA-axis activation under some stressful conditions (Diorio et al., 1993).

CRH projection

CRH is not only a potent activator of the HPA-axis, but CRH is also known to play a role in the central nervous system as a neuromodulator. CRH-containing neurons have been identified throughout the neocortex, with high densities in the prefrontal

and cingulate cortices (Swanson et al., 1983). Furthermore, high densities of CRH-immunoreactivity have been observed in the central nucleus of the amygdala, the BNST, the hippocampus, the nucleus accumbens, the posteromedial thalamic nuclei, such as the mediodorsal nuclei, the substantia nigra, the locus coeruleus, the dorsal and medial raphe nuclei, the periaqueductal grey, the olfactory bulbs, the parabrachial nuclei, the nucleus of the solitary tract and the cerebellum (Merchenthaler, 1984; Morin et al., 1999; Sakanaka et al., 1986; Swanson et al., 1983; Van Bockstaele et al., 1996; Takahashi et al., 1998). Furthermore, CRH has been localised in the adrenal medulla, lymphocytes, testis, pancreas, stomach and small intestine. High levels of CRH have been measured in the plasma of pregnant women, whereas CRH is not detectable under non-pregnant circumstances. The source of CRH in pregnancy appears to be the placenta (Owens and Nemeroff, 1991).

Urocortin

Two non-mammalian homologues of CRH, urotensin and sauvagine, have been described (Lederis et al., 1982; Montecucchi et al., 1980). More recently, a new member of the CRH peptide family was discovered. This mammalian peptide, called urocortin (UCN), was found to bind to CRH receptors with higher affinity than CRH itself (Vaughan et al., 1995; Donaldson et al., 1996). High levels of mRNA of this endogenous neuropeptide have been found in various midbrain areas and the Edinger-Westphal nucleus in the rat brain. Moderate levels have been observed in the hippocampus, the basal ganglia, the medial septum, the medial and cortical amygdaloid nuclei, the PVN, the ventromedial nucleus of the hypothalamus, the superior colliculus, the red nucleus, the substantia nigra pars compacta and the

cerebellar cortex (Wong et al., 1996). Furthermore, UCN containing cell bodies have been identified in the cholinergic laterodorsal tegmental nuclei, the serotonergic dorsal raphe nucleus, the periaqueductal grey and, although only few, in the hypothalamus (Kozicz et al., 1998; Morin et al., 1999). In addition, a dense immunoreactive fiber network but no UCN-immunoreactive neurons were observed in the lateral septum (Kozicz et al., 1998; Morin et al., 1999). Although there seems to be a regional specificity for UCN expression in rat brain, in human brain it has been reported to show a much more uniform distribution (Takahashi et al., 1998). Very recently, urocortin II (UCNII, also known as stresscopin-related peptide) (Reyes et al, 2001; Hsu et al, 2001) and urocortin III (UCNIII, also known as stresscopin) (Hsu et al, 2001; Lewis et al, 2001), two new members of the CRH neuropeptide family, have been described. This peptide binds to CRHR2 only, and is expressed in discrete regions of the rodent brain, including stress related cell groups in the hypothalamus (PVN and arcuate nuclei) and brainstem (locus coeruleus), and partly overlaps with the expression of CRH and Urocortin. The localisation of UCNIII, on the other hand, is distinct from that of CRF, UCN and UCNII. UCNIII is found in the median pre-optic area, the rostral perifornical area, the posterior part of the BNST and the medial nucleus of the amygdala (Lewis et al, 2001).

Urocortin is more potent in stimulating HPA-axis activity compared to CRH in rat. However, there is evidence that CRH, and not urocortin, is the major endogenous regulator of pituitary ACTH secretion under basal conditions or during acute footshock stress (Turnbull et al, 1999; Masuzawa et al, 1999)

CRH Receptors

So far, two CRH receptors, CRHR1 and CRHR2, have been identified (Chang et al., 1993; Chen et al., 1993; Vita et al., 1993; Lovenberg et al., 1995b). Two splice variants are known from CRHR2, CRHR2 α and CRHR2 β (Lovenberg et al., 1995a). CRHR2 β have been observed to be expressed in the brain and in the periphery, with the greatest abundance in the heart and skeletal muscle. CRHR2 α , in contrast, is found almost exclusively in the brain. Likewise, CRHR1 is primarily localised in the brain. CRHR1 and CRHR2 α receptor distribution patterns, however, are partly distinct. Thus, CRHR1 have been almost exclusively observed in frontal cortical areas, the cholinergic basal forebrain (medial septum and diagonal band of Broca), the brainstem cholinergic nuclei (laterodorsal tegmental nucleus and pedunculopontine tegmental nucleus), the superior colliculus, the basolateral nucleus of the amygdala, the cerebellum, the red nucleus, the trigeminal nuclei, and anterior pituitary. CRHR2 α receptors, however, are more strongly expressed in the PVN, the ventromedial nucleus of the hypothalamus, the lateral septum, the cortical and medial nuclei of the amygdala and the serotonergic nuclei, whereas other areas such as the olfactory bulb, the hippocampus, the entorhinal cortex, the BNST and periaqueductal grey show a more mixed expression of CRHR1 and CRHR2 α (Chalmers et al., 1995; Lacroix and Rivest, 1996; Lovenberg et al., 1995b; Sauvage and Steckler, 2001). Furthermore, others have reported additional CRHR1 expression in the central nucleus of the amygdala and substantia nigra (Ambrosio et al., 1997; Lacroix and Rivest, 1996) and in the locus coeruleus, (Sauvage and Steckler, 2001; Sanchez et al., 1999). There is, however, evidence that expression patterns differ between species and even strains (for review see: Steckler, Holsboer, 1999).

On the basis of expression patterns, it has been suggested that UCN might be the endogenous ligand of CRHR2 (Vaughan et al., 1995). However, the recently discovered ligands which have stronger affinity for the CRHR2, UCNII and UCN III, also known as stresscopin in human, may be more likely candidates (Reyers et al., 2001, Hsu and Hsueh, 2001, Lewis et al., 2001).

CRH and Behaviour

Effects of CRH administration on behaviour have been extensively reviewed (Dunn and Berridge, 1990; Owens and Nemeroff, 1991; Heinrichs et al., 1997; Steckler and Holsboer, 1999). Intracereboventricular (i.c.v.) CRH administration increases anxiety-related behaviour in a variety of anxiety tests (Dunn and Berridge, 1990). Similar effects have been found after i.c.v. administration of urocortin (Moreau et al., 1997). These effects seem to be centrally mediated, as peripheral CRH administration does not result in altered anxiety-related behaviour, and anxiogenic effects are still present after CRH administration following pre-treatment with doses of dexamethasone that adequately block HPA-axis activation (Dunn and Berridge, 1990). However, i.c.v. administration results in non-specific stimulation of both CRHR1 and CRHR2. Several techniques have been applied to reveal which receptor subtype mediates the effects on anxiety-related behaviour. Investigations using oligodeoxynucleotides (ODNs) indicated that ODNs against CRHR1 reduced anxiety-related behaviour (Liebsch et al., 1999; Skutella et al., 1998; Heinrichs et al., 1997a), whereas ODNs against CRHR2 α mRNA did not affect anxiety-related behaviour (Liebsch et al., 1999; Heinrichs et al., 1997a).

In addition, two studies, investigating two independently generated CRHR1 knockout mice, show that loss of CRHR1 leads to reduction in anxiety-related behaviour (Timpl et al., 1998; Smith et al., 1998). Subsequently, anxiety-related behaviour in three independently generated CRHR2 knockout mice was investigated. Two groups reported increased anxiety-related behaviour in these knockout animals in some, but not all anxiety tasks (Kishimoto et al., 2000; Bale et al., 2000), whereas Coste and colleagues (2000) did not find a significant genotype dependent effect on anxiety-related behaviour. Further support for a role for CRHR1 subtype in anxiety-related behaviour comes from reports of anxiolytic effects induced by several CRHR1 antagonists (Griebel et al., 1998; Steckler and Holsboer, 1999). However, others reported that the anxiolytic actions of these antagonists only became apparent after CRH administration (Schulz et al., 1996), or after increment of stress in the test paradigm (anxiolytic effects of CRHR1 antagonist treatment in the fear potentiated startle paradigm, but not in acoustic startle paradigm for example). This is in line with a study from Okuyama and colleagues (1999), in which they showed reversal of CRH induced anxiety-related behaviour and anxiolytic effects after stress, but not under basal conditions. These stress-dependent effects of CRHR1 antagonists have also been suggested as a possible explanation for the equivocal results in rats tested on the plus maze (Griebel et al., 1998). Recently, Takahashi and colleagues (2001) showed that acute blockade of CRHR2 using antisauvagine-30 also reduces anxiety-related behaviour in three models of anxiety, which is in contrast to the effects of life-long loss of CRHR2 (see above). In addition, results from recent data suggest that i.c.v. administration of UCNII and UCNIII reduce anxiety related behaviour on the elevated plus maze, but only hours after administration (Reul and Holsboer, 2002). Taken together, this suggests that stimulation of CRHR1 increases anxiety-

related behaviour, whereas the role of CRHR2 in anxiety-related behaviour is less clear.

Increasing anxiety, in general, is accompanied by a change in arousal. This is coupled with a heightened focus of attention towards stimuli perceived as threatening. CRH, however, might modulate arousal and attentional processes independent of the positive and negative value of stimuli.

Animals may be underaroused, optimally aroused or overaroused, and performance is related to arousal in a negative-U-related manner (Yerkes and Dodson, 1908). CRHR1 are found in brain areas such as the cholinergic basal forebrain nuclei, the brainstem cholinergic nuclei, the superior colliculi, frontal cortical areas, locus coeruleus, ventral tegmental area and substantia nigra (Sauvage and Steckler, 2001). These brain areas play an important role in the mediation of attentional and executive functions (Gallagher and Holland, 1994; Garcia-Rill, 1991; Muir et al., 1992, 1994, 1996; Steckler et al., 1994), and have reciprocal connections with the central amygdala, an area which has also been implicated in attentional processes (Gallagher and Holland, 1994; Loughlin and Fallon, 1983). It is of course tempting to speculate that the central amygdala is one of the important sources of CRH input to, at least some, of these areas (Steckler and Holsboer, 1999). This, together with the interactions between CRH and the cholinergic system (see above), a system that is crucial in attentional processes, suggest that CRH might play a role in attention.

Furthermore, CRH is known to have effects on several other aspects of behaviour. Locomotor activity, for example, is affected by CRH administration, although this effect seems to depend on the testing environment. Thus, increased locomotor

activity occurs in familiar environments after CRH treatment, whereas CRH administration reduces locomotor activity under novel conditions (Dunn, Berridge, 1990). In addition, numerous studies show that CRH plays a role in learning and memory, feeding and reproductive behaviour (Dunn, Berridge, 1990).

CRH in depression, clinical findings

When Harvey Cushing described the Cushing syndrome in the early 20th century, he already mentioned a high occurrence of depression in these patients (Cushing, 1913; 1932). Later studies showed that patients diagnosed with Cushing syndrome show a higher occurrence of depression than patients with other pituitary tumours (Kelly et al., 1980). Furthermore, reduction of Hamilton rating scale score, an index for the severity of depression, was found to be accompanied by normalisation of the cortisol plasma levels in these patients (Kelly et al., 1983). This suggests that increased HPA-axis activity and depression may correlate.

ACTH and cortisol plasma levels of patients suffering from depression are often elevated. Moreover, a flattening of circadian cortisol plasma levels was observed (Schmider et al., 1995; Deuschle et al., 1997). Furthermore, the circadian disturbance in ACTH and cortisol plasma levels in depressives normalises during clinical remission (Mendlewicz and Linkowski, 1987). In addition, increased adrenal volumes have been reported in depressed patients (Nemeroff et al., 1992; Amsterdam et al., 1987; Rubin et al., 1995). Likewise, the size of the pituitary has been shown to be increased in depressed patients (Krishnan et al., 1991). Although it has been suggested that adrenal gland enlargement might be a measure of cumulative life time depression (Nemeroff et al., 1992), there is also evidence that increased adrenal

gland size is state dependent, as it has been shown that it reverts to the normal size range during treatment induced remission (Rubin et al., 1995).

It has been hypothesised that CRH hypersecretion is, at least in part, responsible for the hyperactivity of the HPA-axis seen in depression. Results from several studies indicate that CRH levels in the cerebrospinal fluid (CSF) are increased in depressed patients (Risch et al., 1992; Nemeroff et al., 1991, 1984; Banki et al., 1987). Moreover, there is evidence that CRH neurons in the PVN are hyperactive in depressed patients (Raadsheer et al., 1994, 1995). However, others indicated that increased CSF CRH levels could only be demonstrated in depressed patients who were nonsuppressors in the dexamethasone suppression test (DST, see below: Roy et al., 1987). One possibility could be that the level of CRH overactivity is related to the severity of depression, and both factors have indeed been shown to correlate (Risch et al., 1992).

Administration of dexamethasone, a glucocorticoid agonist, can suppress HPA-axis activity in controls, whereas it is less effective in depressed patients, which was first described by Carroll and co-workers (Carroll et al., 1968). This effect became known as the dexamethasone suppression test (DST). Furthermore, in healthy controls, dexamethasone is sufficiently potent to inhibit CRH-induced ACTH and cortisol stimulation (von Bardeleben et al., 1985), whereas ACTH and cortisol are released after CRH administration despite dexamethasone pre-treatment in depressed patients (Holsboer et al., 1987; Holsboer-Trachsler et al., 1991). Successful treatment has been reported to result in cortisol levels indistinguishable from that of controls, whereas ACTH levels were still enhanced (Holsboer-Trachsler et al., 1991). CRH

administration in depressives results in a blunted ACTH response, but not in cortisol response, compared to healthy controls (Holsboer et al., 1986). However, others reported that only DST nonsuppressors exhibit a blunted ACTH response to CRH, whereas DST suppressors exhibit a normal ACTH response to CRH (Krishnan et al., 1993). Krishnan and co-workers suggested that these two abnormalities in HPA-axis function are functionally related, and therefore may be due to similar pathophysiological mechanisms. Furthermore, elevations of CSF CRH levels in depressed patients were normalised after ECT, suggesting that the increase in CSF CRH concentrations in depressed patients is state dependent (Nemeroff et al., 1991).

Taken together, dysregulated HPA-axis functioning in depression is, at least in part, likely to be due to increased CRH secretion. Furthermore, increased CRH levels in the CSF indicate that the hyperactivity of the CRH system is not limited to an augmented release of CRH into the portal vein, but possibly also in the brain. Therefore, it is possible that some of the symptoms, such as increased anxiety and reduced attentional performance, observed in depression, are due to a hyperactive central CRH system. This could be due to direct effects of CRH, or via alteration of other neurotransmitter systems.

Interactions of CRH and the serotonergic system

Occurrence of depression is not only associated with alteration in the CRH system. In particular, the serotonergic (5-hydroxytryptamine, 5-HT) system has been implicated in the modulation of anxiety and depression, especially the 5-HT_{1A} receptor (Deakin, 1998; Heinrichs et al., 1997b; Holsboer and Barden, 1996). There are nine clusters of serotonin containing cells in the brain (B1→B9). Serotonergic

neurons projecting to the forebrain are primarily located within the pons structures, the dorsal (B6 and B7) and median (8) raphe nuclei and B9. The other clusters (B1→B5) project to the brainstem and spinal cord. 5-HT_{1A} receptors are expressed presynaptically, where they function as inhibitory autoreceptors, and postsynaptically. Systemic administration of 5-HT_{1A} agonists can inhibit the firing rate of serotonergic neurons in the dorsal raphe nucleus (Sprouse and Aghajanian, 1987) and has been reported to inhibit central 5-HT release (Hjorth and Sharp, 1991).

Interactions between the serotonergic system and CRH can be found at various levels. It has been shown that CRH can affect serotonergic function via actions at the level of the cell body region of the dorsal raphe serotonergic system (Kirby et al., 2000; Price et al., 1998) and serotonergic responsivity is altered after long-term i.c.v. infusion of CRH (Linthorst et al., 1997). Furthermore, CRH can affect the serotonergic system indirectly via HPA-axis activation. For example, glucocorticoid hypersecretion may downregulate the sensitivity of postsynaptic 5-HT_{1A} signal transduction or downregulate the expression of 5-HT_{1A} receptors (Chaouloff, 1993; Deakin et al., 1990). Furthermore, serotonergic hyperresponsivity following exposure to a psychological stressor has been reported in mice with impaired GR (Linthorst et al., 2000). Conversely 5-HT is known to influence HPA-axis activity via induction of CRH release. Serotonergic neurons have been shown to synapse at CRH-containing fibers in the PVN (Sawchenko et al., 1983; Liposits et al., 1987). More specifically, 5-HT_{1A} receptors seem to play a role in regulating CRH release. Systemic administration of 8-hydroxy-2-(di-n-propylamino)tertalin (8-OH DPAT), a 5-HT_{1A} receptor agonist, activates the HPA-axis (Calogero et al., 1990). Furthermore, local application of 8-OH DPAT into the hypothalamus, or more

specifically, into the PVN also increases HPA-axis activity (Korte et al., 1991; Pan and Gilbert, 1992). The 8-OH DPAT-induced increase of ACTH is inversely correlated with a reduction of hypothalamic CRH. Moreover, 8-OH DPAT induced ACTH release can be diminished by CRH antiserum (Calogero et al., 1990), suggesting that activation of the HPA-axis by 8-OH DPAT occurs, at least in part, via CRH release triggered off by stimulation of 5-HT_{1A} receptors at hypothalamic level. In addition, ipsapirone (another 5-HT_{1A} agonist) also induces corticosterone release, and this effect is abolished after lesion of the PVN (Bagdy, 1996).

5-HT also plays a role in sleep, appetite-regulation, cognitive function, impulsivity, sexual behaviour, motor function and modulation of limbic/affective responsiveness. Furthermore, 5-HT is involved in neuronal homeostasis, and in regulation of the HPA-axis (Ressler and Nemeroff, 2000). In depression, most pharmacological evidence supports the hypothesis that the serotonergic system is hypoactive (Maes and Meltzer, 1995). In addition, dysfunction of the serotonergic system has been reported to occur in patients suffering from anxiety disorders. It has been suggested that this dysfunction mediates the symptoms of both disorders, although it is less likely that the underlying causes of these disorders could be found in the 5-HT system per se (Ressler and Nemeroff, 2000): antidepressants are effective in both treatment of major depression and some anxiety disorders. However, it requires several weeks of treatment before clinical improvement can be observed (Blier et al., 1990; Charney et al., 1990). Data from preclinical studies show that long-term, but not acute, treatment with SSRI's enhances 5-HT neurotransmission (Chaput et al., 1986). Furthermore, increased density and sensitivity of the postsynaptic 5-HT_{1A} receptor has been shown after repeated antidepressant treatment or electroconvulsive

shock therapy (ECT) (de Montigny and Aghajanian, 1978; Dijcks et al., 1991). This opens the possibility for an important role of the 5-HT_{1A} receptor in the treatment response, although it is not clear at this moment whether main effects are mediated at pre- and/or postsynaptic level. Thus, data from studies investigating presynaptic 5-HT_{1A} function indicate that long-term antidepressant treatment desensitises 5-HT_{1A} autoreceptors function (Chaput et al., 1986; Hervas et al., 2001; Invernizzi et al., 1994; Le Poul et al., 1995), and it has been suggested that effects of chronic antidepressant treatment on pre- or post-synaptical binding sites might be dependent on the class of antidepressant used (Welner et al., 1989).

This evidence has led to the hypothesis that anti-anxiety or antidepressant effects of antidepressants might exert their clinical action via enhancement of serotonergic neurotransmission caused by desensitisation of 5-HT_{1A} autoreceptors (Albert et al., 1996), possibly in concert with the increased postsynaptic sensitivity of 5-HT_{1A} receptors (Welner et al., 1989). However, interactions with other 5-HT receptor subtypes, or other neurotransmitter / neuromodulator systems, such as CRH, are likely to contribute as well.

Interactions of CRH and the cholinergic system

Another candidate neurotransmitter system via which CRH may effect behaviour is the cholinergic system. One of the properties of CRH is to potentiate arousal (Koob et al., 1990). Arousal can be defined as a state which optimises the processing of sensory stimuli in the cerebral cortex (Hebb, 1955) and is therefore related to attentional function (Robbins and Everitt, 1994). This in turn suggests that CRH plays a more general role than just to mediate responses to aversive events, but

maintains attention towards events or cues of biological significance, regardless of the nature of the reinforcer (Merali et al., 1998). It could be speculated that activation of the CRH system is beneficial for optimising attentional processes, whereas a hyperactive CRH system might be disruptive. Effects of CRH on attentional processes might be via interactions with the cholinergic system.

There are several findings to support this hypothesis. For example, CRHR1 is highly abundant in brain areas which have been suggested to play a role in attention, such as frontal cortical areas, the superior colliculi, the cholinergic basal forebrain and brainstem nuclei (Bittencourt and Sawchenko, 2000; Chalmers et al., 1995). Furthermore, a high degree of co-localisation of choline-acetyltransferase and CRH receptor-like immunoreactivity has been observed in both the murine basal forebrain and brainstem, with the exception of the substantia innominata (Sauvage and Steckler, 2001). The prefrontal cortex (Muir et al., 1996), the superior colliculi and the cholinergic basal forebrain (Muir et al., 1992; 1994; Robbins et al., 1989) have been strongly implicated in the mediation of different types of attention, and the cholinergic brainstem nuclei have been also demonstrated to control arousal and attentional functions (Garcia-Rill, 1991; Steckler et al., 1994). Moreover, in rats, i.c.v. CRH administration produces moderate to strong stimulation of Fos expression within the basal forebrain and brainstem nuclei, i.e., within the medial septum and diagonal band of Broca, the laterodorsal and the pedunculopontine tegmental nuclei, while only relatively weak Fos expression is induced within the substantia innominata (Bittencourt and Sawchenko, 2000). Furthermore, i.c.v. but not peripheral injections of CRH increases hippocampal acetylcholine release (Day et al., 1998a).

However, CRH might affect attentional processes via other neurotransmitter system such as the noradrenergic system. It has been demonstrated that the locus coeruleus is also involved in modulation of arousal and attention (Carli et al., 1983; Cole and Robbins, 1992; Foote et al., 1983; Usher et al., 1999). Electrophysiological activation of the noradrenergic locus coeruleus by CRH has been reported (Butler et al., 1990; Valentino et al., 1993), opening the possibility that this may be an additional site through which CRH exerts influence on attentional abilities. Interestingly, there is evidence for strong CRHR1 immunoreactivity co-localised with tyrosine hydroxylase at the level of the locus coeruleus (Sauvage and Steckler, 2001). Taken together, these observations support the idea that CRH might play a prominent role in the modulation of attentional processes, possibly through activation of CRHR1. However, behavioural evidence is lacking.

Anxiety in depression

From clinical investigations, we know that the CRH system is disturbed in depressed patients. It is less clear whether these disturbances are the cause or the result of depressive illness.

Even if CRH is causally involved, disruption of the CRH system may only lead to a limited set of depressive symptoms. Therefore, investigation of the role of the CRH system in different behavioural processes linked to depression is desirable. However, depression is hard to model in animals. Although a number of tests have been described in which effects of antidepressants can be measured in animals, they do not necessarily reflect good models of depression with high validity. Employing a range

of paradigms might therefore be advisable for investigation of several phenomena that are disturbed in depression.

One of the frequently occurring symptoms in depression is increased anxiety. Depression and anxiety disorders are generally classified as separate types of syndromes, however, many of the symptoms are overlapping in these diseases including fatigue, impaired concentration, irritability, sleep disturbance, worry and restlessness (Ninan, 1999). In addition, these syndromes share a common pathophysiology (Weiss et al., 1994). Furthermore, there is a high occurrence of anxiety disorders in depressive patients and vice versa (Ressler and Nemeroff, 2000).

Anxiety-related behaviour in animals

Exposure to a foot shock avoidance procedure or just to an elevated plus maze induced Fos-like immunoreactivity in the medial prefrontal, cingulate and ventrolateral orbital cortices, taenia tecta, nucleus accumbens, PVN, medial nucleus of the amygdala and lateral septum (Duncan et al., 1996). Exposure to airpuff stimuli that produced ultrasonic vocalisations induced Fos-like immunoreactivity to a more limited extent compared to shock avoidance and exposure to an elevated plus maze, with only the medial prefrontal cortex, medial nucleus of the amygdala and lateral septum being affected. Common forebrain regions affected after all three procedural manipulations were the medial prefrontal cortex, the medial amygdala and the lateral septum. Therefore, it has been suggested that these three regions are components of a common circuit related to anxiety or distress (Duncan et al., 1996). However, it is also evident from these results that there are task-specific alterations in brain activity. For example, expression of Fos-like immunoreactivity in the PVN and locus

coeruleus is increased after foot shock avoidance procedure, but not after elevated plus maze or air puff procedures, although others did find an increased after plus maze behaviour in the PVN (Silveira et al., 1993). Lack of Fos-like immunoreactivity in the PVN was also reported after conditioned fear, whereas conditioned fear did increase Fos-like immunoreactivity in, among other areas, medial prefrontal cortex, various amygdalar nuclei and the lateral septal nucleus (Beck and Fibiger, 1995).

This is in line with findings showing that different brain structures are involved in different types of anxiety-related behaviour. For example, a dissociation between trait and state anxiety has been suggested (Griebel et al., 1993; Beuzen and Belzung, 1995). Anxiolytics of the GABA/benzodiazepine and serotonergic class have been reported to reduce anxiety-related behaviour in a light-dark choice test, a model that has been suggested to allow investigation of state anxiety. Conversely, anxiolytics of the GABA/benzodiazepine class, but not of the serotonergic class, reduce anxiety-related behaviour in a free exploration paradigm, a model for trait anxiety (Kopp et al., 1999). Likewise, it has been demonstrated that selective cholecystokinin type B receptor agonists reduces state, but not trait anxiety, measured in these paradigms (Belzung et al., 1994b). The anxiolytic effects of 5-HT_{1A} agonists have also been demonstrated to be test specific (Griebel et al., 2000). In addition, it has been claimed that drugs with proven clinical efficacy in panic disorder have reliably stronger effects on flight responses than on other components of the defensive repertoire (Rodgers, 1997). This indicates that various neurotransmitter systems play different roles in the modulation of anxiety depending on the type of anxiety-related behaviour being assessed.

To complicate matters, anxiolytic effects of benzodiazepines in different anxiety tasks seem to be dependent on brain area. Local administration of midazolam, an anxiolytic of the benzodiazepine class, into the septum increased open arm exploration on the elevated plus maze and decreased burying behaviour in the shock-probe test, both indicative for anxiolytic action. When midazolam was administered in the amygdala, it did not affect these parameters. However, injection of midazolam into the amygdala impaired shock-probe avoidance, an effect that did not occur after septal infusions (Pesold and Treit, 1994). A follow-up experiment of the same group showed that these impairment in shock-probe avoidance can be induced by infusion of midazolam in the central, but not in the basolateral nucleus of the amygdala (Pesold, Treit, 1995), which indicates that the shock-probe avoidance effects of midazolam are likely to be mediated by the central nucleus of the amygdala. Strikingly, basolateral infusion increased open arm activity on the elevated plus maze, but left shock-probe burying behaviour unaffected. This is in line with amygdaloid nuclei-dependent effects of benzodiazepines on plus maze behaviour by Green and Vale (Green and Val, 1992). Further support for a specialised role of distinct nuclei of the amygdala comes from a fear conditioning study. Here, it was shown that lesioning the central nucleus of the amygdala or basolateral amygdala prevented distinct lesion-specific behavioural responses elicited by a conditioned stimulus (Killcross et al., 1997).

Taken together, there is evidence that different aspects of anxiety-related behaviour can be assessed in rodents. These aspects may be mediated via different brain structures / pathways. However, there is also evidence that some areas mediate

symptoms across different anxiety disorders. Rauch and colleagues (1997), for example, showed in a positron emission tomography study that elements of the paralimbic belt (i.e., posterior medial orbitofrontal, anterior cingulate, insular, parahippocampal and anterior temporal cortex), and right inferior frontal cortex mediate symptoms across different anxiety disorders in man.

Experimental approaches to study anxiety-related behaviour

In this work, a range of tests was used in order to assess anxiety-related behaviour. Conceptually, the paradigms used in this work can be grouped into different categories, such as tasks primarily based on free exploration of novel environments (elevated plus maze, light dark exploration, hole board), free exploration of novelty, but based within a familiar surrounding (novel object exploration), conflict paradigms (Vogel conflict) and tasks involving a strong mnemonic component (fear conditioning).

The elevated plus maze has been suggested to be based on the natural aversion of rodents for heights and open spaces (Montgomery, 1955), and can be considered a standard paradigm for testing anxiogenic- and anxiolytic-like responses in rats and mice (Lister, 1987). The main anxiogenic factor suggested to underlie explorative behaviour in the light dark exploration is the aversive nature of an open, brightly illuminated area (Costall et al., 1989; Crawley and Goodwin, 1980; Crawley, 1981). Explorative anxiety paradigms like these have been suggested to measure state anxiety (Belzung and Le Pape, 1994a; Belzung et al., 1994b; Kopp et al., 1999). In contrast, in the novel object exploration task, mice are first habituated to an environment and subsequently exposed to novelty (Misslin and Ropartz, 1981).

Exploration of novelty from an area known to be safe has been suggested to fundamentally differ from exploration of a totally new environment and has been claimed to reflect trait rather than state anxiety (Griebel et al., 1993).

The hole board, on the other hand, has been suggested to allow investigation of exploratory behaviour in rats (Escorihuela et al., 1999) and mice (Rogers et al., 1999). In mice, it has been shown that the number of hole visits decreases following restraint stress, which is prevented by treatment with benzodiazepine and non-benzodiazepine anxiolytics, independent of locomotor activity (Takeda et al., 1998).

In addition, analysis of different aspects of spontaneous behaviour has been suggested to be indicative of anxiety-related behaviour. For example grooming behaviour, which has been shown to increase after stress exposure in a range of paradigms, has been considered as displacement behaviour (Dunn et al., 1981; van Erp et al., 1994; Spruijt et al., 1992). In addition, it has been demonstrated to increase after i.c.v. CRH administration (Britton et al., 1986; Diamant, de Wied, 1993; Sherman, Kalin, 1986), independently of HPA-axis activation (Morley and Levine, 1982).

Conflict behaviour can be assessed by several procedures, and is also believed to reflect anxiety-related behaviour. In the present set of studies, the Vogel conflict test was chosen. In this paradigm, water deprived animals receive a mild electric shock during drinking. The test has been shown to be sensitive to various anxiolytics, but not to a number of other psychoactive drugs, such as d-amphetamine, and scopolamine (Vogel et al., 1971). The advantage of this test, compared to other

conflict tests, is that it allows one-trial measurements, whereas others require training. It has been shown that, as in conditioned fear (see below), the amygdala plays an important role in conflict behaviour (Moller et al., 1994).

Conditioned fear was employed as a test in which remembrance of a stressful situation plays a role. The key brain area involved in conditioned fear is the amygdala. Damage to this area greatly changes the way animals and people act in the face of danger. It has been proposed that this fear system of the brain is involved in at least some anxiety disorders (LeDoux, 1998; Öhman, 2001). Several brain areas, which appeared to be involved in conditioned fear, have been suggested to play a role in some anxiety disorders, which occur in man. For example, damage to the hippocampus interferes with conditioning to contextual cues (Phillips and LeDoux, 1992; Kim and Fanselow, 1992; Selden et al., 1991; Blanchard et al., 1970). It has been suggested that generalisation to fear, as is seen in some anxiety disorders, might be due to weakening of contextual constraints on fear (LeDoux, 1998). This is consistent with the fact that stress impairs the anatomy and physiology of the hippocampus and behavioural aspects in which the hippocampus is believed to play a role (Sapolsky, 1996; McEwen and Sapolsky, 1995). In addition, damage to the medial prefrontal cortex results in a prolongation of extinction of the conditioned response (Morgan et al., 1993; Morgan, LeDoux, 1995). Thus, fear that is established is difficult to get rid off, again a similar to what is seen in some anxiety disorders. Furthermore, prefrontal cortex functioning is also known to be affected by stress (Diorio et al., 1993).

5-HT_{1A} stimulation

A simple model for assessing 5-HT_{1A} autoreceptor function has been described by Bill and colleagues (1991). Administration of a range 5-HT_{1A} receptor agonists induces hypothermia in rodents. In mice it is believed to be due to stimulation of presynaptic autoreceptors whereas in rats it appears to be due to postsynaptic receptor stimulation (Bill et al., 1991; Martin et al., 1992). 8-OH DPAT induced hypothermia in mice is attenuated after long-term treatment with antidepressants of various classes, as well as by repeated ECT (Bill et al., 1991; Goodwin, 1989), indicating desensitisation of 5-HT_{1A} autoreceptor function. This is in agreement with studies using other techniques such as microdialysis or single cell recording (Chaput et al., 1986; Hervás et al., 2001; Invernizzi et al., 1994; Le Poul et al., 1999). However, the attenuation of the hypothermic response is not entirely specific for antidepressant treatment. Furthermore, 8-OH DPAT induced hypothermia can also be antagonised by D2 antagonists, suggesting a role for dopamine D2 receptors in this phenomenon (Martin et al., 1992).

Attentional processes and depression

Patients suffering from major depression also display attentional deficits (Mialet et al., 1996; Rief and Hermanutz, 1996; Lemelin et al., 1997; Williams et al., 2000). These attentional deficits have been reported to be particularly marked in patients with psychomotor retardation, whereas non-retarded depressed patients have fewer attentional disturbances. Different attentional deficit patterns in clinical depression are reported, such as distractor inhibition disturbance and deficiency in processing resources, which occurs in different depressive subgroups. (Lemelin and Baruch, 1998). In fact, the emotional and the cognitive aspects of the disorder are not

independent, and it may be suggested that the increased anxiety often observed in depression is in part related to attentional dysfunction: given that CRH increases arousal, patients may have problems filtering relevant from irrelevant environmental stimuli, and therefore have difficulties in monitoring and amending their behaviour to the demands of the environment. This in turn may lead to a bias in the perception of stimuli as being potentially threatening, with consequently increased anxiety.

Attentional processes in animals

Many behavioural procedures have been proposed for modelling attention in animals. Recently, Bushnell categorised these tasks in orienting, expectance, stimulus differentiation, sustained attention and parallel processing (Bushnell, 1998). In this work, attentional processes are assessed with the 5-choice serial reaction time task (5-CSRTT), one of the most well characterised stimulus selection models for animals (Bushnell, 1998). This task has analogies with a range of human attentional paradigms (Mirsky and Rosvold, 1960; Sahakian et al., 1993; Wilkinson, 1963), and provides measures of sustained and divided attention in temporal and spatial domains (Bushnell, 1998; Muir et al., 1996; Robbins et al., 1993): the animal has to continually monitor an array of five possible stimulus locations for the occurrence of a brief visual stimulus (sustained attention), has to distribute its responding across this array (spatially divided attention) and to respond to repeatedly presented light stimuli.

The paradigm was originally developed by the group of Robbins (Carli et al., 1983) for testing rats. Recently, it has been demonstrated that attentional processes could be assessed in mice in an adapted 5-CSRTT set-up (Humby et al., 1999), and that attentional performance can be manipulated by the same mechanisms as described

for manipulating performance in rats (Muir et al., 1994; Bushnell et al., 1997), monkeys (Voytko et al., 1994) and humans (Wesnes and Revell, 1984a; Wesnes and Warburton, 1984b).

The use of mouse mutants to study behavioural effects of chronic alterations in the CRH system

The use of genetically engineered mice has several advantages. Firstly, receptors can be specifically inactivated which, for several receptors, cannot be done with the use of antagonists. Secondly, these techniques enable the possibility to study the effects of overproduction of a peptide, without exposing animals to surgery. Thirdly, effects of long-term loss or overproduction of peptides can be assessed. However, there are also some disadvantages of the use of mutant animals. Altering gene products in a mutant mouse may lead to a cascade of effects, which may make it difficult to predict direct effects of alteration of gene expression and phenotype (for example, Holmes, 2001). Therefore, the use of mutant animals should be considered as an addition to pharmacological methods, and not as a replacement.

Outline and scope of this thesis

The aim of the experiments presented in this thesis is to investigate the role of CRH in several behavioural and neuroendocrinological phenomena that are known to be disturbed in patients suffering from depression and can be assessed in rodents. In order to exploit the advantage of molecular engineered selective inactivation or overproduction of genes, the mouse was chosen as study object.

First, it was investigated whether test results from one anxiety paradigm would be predictive for the outcome of another, based on different psychological principles, and whether these effects would be strain specific. These data could then be used for choosing an appropriate mouse strain in studies in which these tasks were applied. Next, it was investigated whether long-term treatment with citalopram, a compound that has been shown to be effective in the treatment of depression as well as anxiety disorders, would affect anxiety-related behaviour in mice.

On basis of these results, it was hypothesised that, in order to investigate the pathophysiology of anxiety, subjects with increased emotionality are needed. Therefore, mice overexpressing CRH were chosen. In Chapter 5, the anxiety-related behaviour of these animals in a range of anxiety paradigms is presented.

Furthermore, it was investigated whether the neuroendocrinological profile of these animals would show analogies with that of patients suffering from depressive or anxiety disorders. Therefore, HPA-axis activity was studied, both under basal conditions and after pharmacological challenges, which is presented in Chapter 6.

Next, it was investigated whether the effects of citalopram would be different in CRH overexpressing mice compared to wildtype littermates (Chapter 7).

These results indicated that overproduction of CRH leads to an increase in some aspects of anxiety and to a disturbed neuroendocrinological profile. Next, it was investigated whether CRH overproduction would affect sustained and divided

attention. In Chapter 8, the results of excessive CRH production on these attentional processes are presented.

Subsequently, the effects lack of CRH on 5-CSRTT performance was studied. However, lack of CRH does not implicate that CRHR cannot be activated, as several other endogenous peptides are capable of activating CRH receptors (see above). Therefore, in Chapters 10 and 11, data are presented from experiments studying the impaired function of CRHR1 or CRHR2 on visuospatial attentional processes.

Chapter 2

Material and methods

Animals

In all experiments, animals were singly housed in type II cages (207 x 140 x 265 mm) and kept under 12:12 h light-dark cycle (lights on 6.00h), with food and water *at libitum* unless mentioned differently in the specific paradigm descriptions. All testing took place during the light phase of the light-dark cycle, except for the elevated plus maze test, which was performed during the early dark phase. The experiments were performed in compliance with the guidelines of the European Communities Council Directive of 24 November 1986 (86/609/EEC). The following paradigms were used in the different studies:

Examination of spontaneous behaviour

Spontaneous behaviour was analysed in the home cage. Shortly before testing, the animal was aroused by placing it in a confinement for 1 min in order to ensure a certain level of behavioural activation. During this time, the bedding in the home cage was shuffled around and evenly redistributed. A clear plastic lid was placed on top of the cage that prevented escape of the animal but allowed video recording, which started as soon as the mouse was returned to its home cage and lasted for ten minutes. For measurements of locomotor activity, distance travelled was analysed by a video tracking system (VideoMot2, TSE, Bad Homburg, Germany). When

videotracking was not possible due to insufficient difference in contrast between the animals and background, measuring the number of line crossing was used to assess locomotor activity. This was measured by dividing the bottom of the cage virtually in 3×2 equally sized fields, after which the number of line crossings was scored. The times spent rearing, grooming and digging in the bedding material were scored and were calculated as a percentage of the total session time. Digging behaviour was included because pilot studies in which male C57Bl/6 N mice were tested in a marble burying paradigm, indicated that these mice dig in the bedding material, independent of whether marbles were present in the cage or not. Video analysis indicated that when marbles got buried, it happened accidentally by general digging in the bedding material. Data from an additional pilot test, without the presence of marbles, but performed as described above, indicated that acute treatment with diazepam, 20 min before testing, tended to reduce digging behaviour, in male C57BL/6 N mice ($n = 10$). Repeated social defeat increased digging behaviour, which could be significantly prevented by diazepam treatment (Figure 2.1). No effect of diazepam was found in terms of rearing. Takeda and colleagues (1998) showed that rearing decreases in concert with the distance travelled when diazepam is administered in the high dosage. This suggests that the reduced digging in the social defeated groups is not due to sedative effects. Taken together, analysis of digging behaviour might provide additional information on anxiety-like behaviour, although more experimental data is necessary to interpret this parameter.

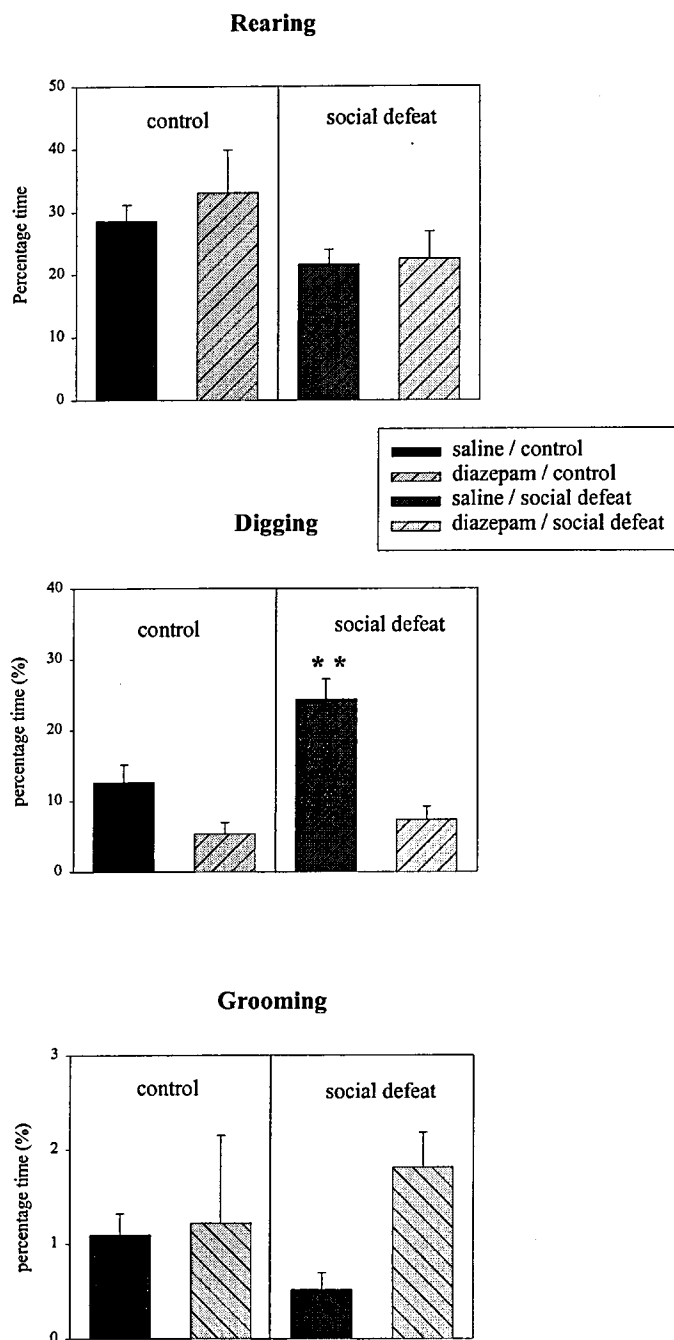


Figure 2.1 Effect of acute diazepam treatment and repeated social defeat in the spontaneous behaviour paradigm. Data is presented as means, with errors bars denoting S.E.M. * * $p < 0.010$ compared to all other groups.

Elevated plus maze

The maze was made of dark grey plastic and was elevated 72 cm above the floor. Four maze arms (28×5 cm) originated from a central platform (5 cm square), effectively forming a cross. Two of these arms (closed arms), located opposite to each other, were enclosed by clear Perspex walls (15 cm high), while the other two arms (open arms) were equipped with a small threshold (0.5 cm high) only, which was fixed at the edges of the arms. Testing took place during the early dark phase. Indirect dim illumination (< 1 lux) was provided by a red light. A test session started by placing the animal on the central platform, facing the enclosed arm. Over a period of 5 min, exploration was video recorded. Between sessions, the maze was thoroughly cleaned with water and dried with paper towel. Subsequently, the number of open and closed arm entries (an arm entry was counted when four paws on an arm), and the time spent in the different compartments of the maze (central platform, open and closed arms) were scored.

Light dark exploration

Testing took place in four chambers (Coulbourn Instruments, Allentown, USA), which allowed testing four animals in parallel. Each chamber was divided into two equally sized compartments ($26 \times 13 \times 38$ cm high each). One compartment was made of clear plastic walls and was illuminated by bright light (500 lux), while the other compartment was made of black plastic, not illuminated and covered by a black roof. An opening, 7.5×7.5 cm wide, connected the two parts of the box. Two infra-red sensor rings (sensor spacings 1.52 cm) allowed measurement of vertical and horizontal activity (sampling frequency 4 Hz), and were connected to a computer equipped with the True Scan Software Vers 1.1A (Coulbourn Instruments). Each

box, including its sensor rings, was surrounded by an additional box ($47 \times 47 \times 38$ cm, white plastic), which prevented the animals from seeing each other.

A session lasted 5 min. From the raw data, the distance travelled and the number of rears made over the 5-min period was calculated. Furthermore, moving time, entries, and relative distance travelled and the relative number of rears in the illuminated compartment was calculated.

Open field

Animals were tested in a square open field ($26 \times 26 \times 38$ cm high, 500 lux illumination), made of a white floor and clear plastic walls, and equipped with infrared photocell sensors. The field was surrounded by an additional box ($47 \times 47 \times 38$ cm high, white plastic). Horizontal and vertical activation was measured using infrared sensor rings and Truscan software version 1.1A (Coulbourn Instruments). Furthermore, exploration of the centre of the open field (18.4×18.4 cm) was assessed .

Novel object exploration

The response to the introduction of a novel object placed in a familiar environment was investigated. First, mice were habituated to an open field for thirty min ($26 \times 26 \times 38$ cm high, 500 lux illumination). Subsequently, animals were briefly returned to their home cage and an object (a bolt, M 12×60 mm high) was placed into the centre of one of the quadrants of the field, after which the mouse was returned for an additional 15 min. During the habituation stage, the distance travelled was measured in 3-min time bins. Furthermore, the number of entries and the time spent in the

quadrant which would contain the object was measured during the last 15 min of the habituation stage and taken as baseline. The same measures were taken once the object had been introduced, and the number of entries and time spent in the object quadrant relative to baseline were calculated. This procedure corrects for preference for a particular quadrant in the testing box. In addition, the total time spent in the object quadrant was measured during this stage.

Vogel conflict task

Testing took place in a grey plastic chamber (20.5 × 20.5 × 30 cm, < 1 lux illumination) equipped with a metal grid and a waterspout, which was located in the centre of one of the walls, 1 cm above the grid. The spout and the grid were connected to a drinkometer and a shock generator (Coulbourn Instruments), which allowed to deliver a 0.15 mA shock over 10 ms. Prior to testing, animals were water deprived for 24 hours. A session started after the animal made 20 licks on the spout to ensure knowledge about the position of the spout prior to start, and terminated after 20 min. Every twentieth lick, the mouse received a shock. Animals failing to make at least 20 responses necessary to start the session within 20 min were excluded. The latency to start the session and number of responses made during the session were registered by a computer.

Hole board exploration

Mice were tested in the same square boxes used for novel object exploration (500 lux illumination), except now a metal floor was inserted, containing sixteen holes (2.2 cm diameter) evenly distributed over the floor (4 × 4 holes). Animals were placed in a corner of the field and allowed to freely explore for 30 min. Distance travelled,

rearing and holes visited were monitored by three infra-red sensor rings connected to a computer equipped with the True Scan Software Vers 1.1A (Coulbourn Instruments).

Conditioned fear

Two chambers were used. One chamber (the conditioning chamber) was identical to the box used for the assessment of the novel object and hole board exploration, except that now a shock grid was placed on the floor, and that the chamber was dimly illuminated (1 lux). The shock grid was connected to a shock generator (Coulbourn Instruments), which allowed delivery of a 0.35 mA shock over 2s time period. The other chamber (the test chamber) was similar, except that instead of a shock floor, the box was equipped with a white plastic floor plate. The shock grid was cleaned with 70% ethanol while the white plastic floor was cleaned with a lemon-flavoured detergent after each animal in order to change the olfactory properties of the two chambers. A light source, placed on top of the chambers, allowed to present a flashing light (26 lux at floor level, flash rate 5 Hz).

A mouse was placed into the centre of the conditioning chamber. After five min of free exploration, a series of three light-shock pairings was delivered, once every 100 sec, with the unconditioned stimulus (US, shock) always presented during the last two seconds of conditioned stimulus (CS, light) presentation (15 sec). Twenty-four hours later, the mice were re-exposed to the test box. After five min of exploration, the CS was presented for a period of five min, but no shock was delivered. The first five minutes of exploration on day 2 were analysed in order to assess when animals reached stable baseline in terms of distance travelled and the time not moving, the

latter being defined as the cumulative time during which an animal did not change its position within a sample time of 250 ms. Therefore, the mean distance travelled and the mean time not moving over this period were taken as baselines. Conditioned suppression of activity, i.e., the distance travelled and the time not moving relative to baseline were taken as indicators for fear conditioning. Automated measurement of the conditioned suppression of ongoing spontaneous behaviour has been shown to be a reliable measure of fear conditioning (Crestani et al., 1999; Killcross et al., 1997), and previous studies have shown a high correlation between freezing scored

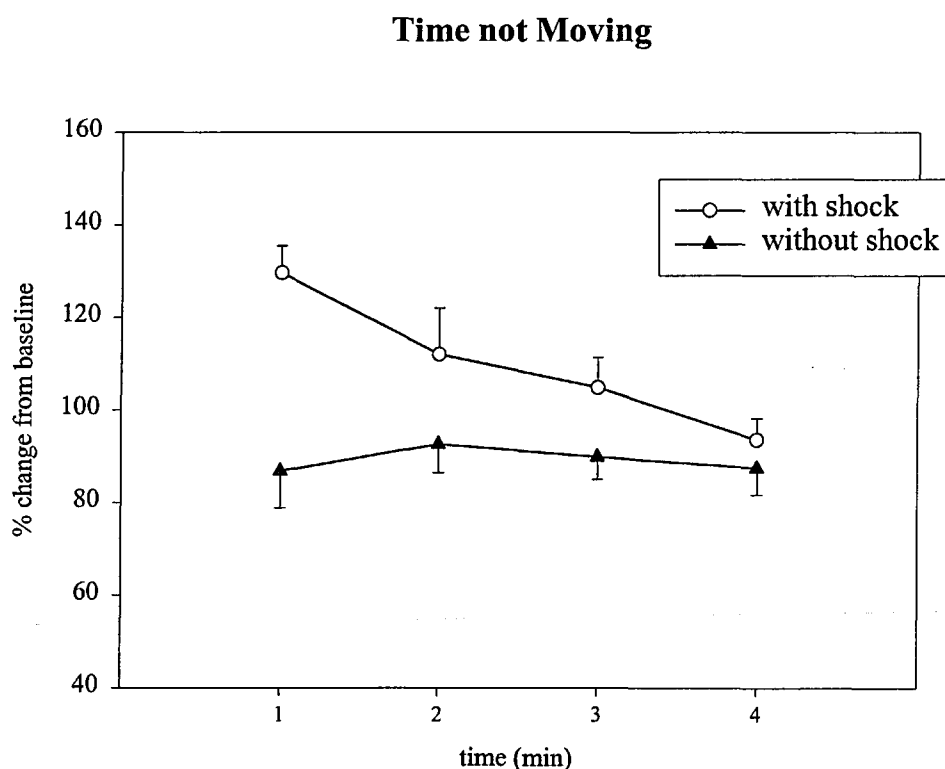


Figure 2.2 Effect of re-exposure to the CS, 24 h after training with or without US. Data is presented as means, with error bars denoting S.E.M.

manually and automated measurement of locomotor activity in mice (Radulovic et al., 1998; Steckler et al., unpublished results) and rats (Bouton and Bolles, 1980). Therefore, time not moving was used.

In order to examine whether the US is not inhibiting activity by it self, a pilot study was performed. Male C57BL/6 N mice (n = 10) were tested according to the above described protocol (shocked group), whereas another group was tested following the same protocol, without presentation of the US (non shocked group). In the shocked group, re-exposure to the CS resulted in suppression of activity, whereas this was absent in the non shocked group (Figure 2.2).

Hot plate

Pain threshold was investigated in the hot plate test. Here, an animal was placed on a square plate (27.5 × 22.5 cm), heated to 55° C. Latency to feet licking or jumping, whatever occurred first, was scored by visual observation.

Rotarod

A rotarod (diameter of rod 3 cm), mounted horizontally 25 cm above a platform, was used in order to examine sensorimotor skills. Plastic dividers (perpendicular to the rod and spaced 10 cm apart) prevented mice from escaping. A trial started by placing an animal on the stationary rod. Each mouse was then required to balance on the rotating rod (3 rpm). A trial terminated either if the mouse fell off the rod or after 5 min. Each animal had three sessions, each spaced with 2 hours.

Inescapable swim stress

A clear plastic cylinder (11 cm in diameter, 25 cm high) was filled to a depth of 7.5 cm with water (25° C). Animals were placed in the water for 6 min. Behaviour was video recorded and immobility time during the last three min of testing was scored by visual observation after testing.

8-OH DPAT induced hypothermia

Animals were weighed and rectal temperature was recorded over 4 seconds (probe length = 2 cm, Thermo-electra, Pijnacker, The Netherlands). Immediately afterwards, animals received a subcutaneous (s.c.) injection of either 8-OH DPAT (0.5 mg/kg) or saline and were returned to their homecage for 20 min, followed by another measurement of rectal temperature.

5-choice serial reaction time task (5-CSRTT)

5-CSRTT: Apparatus

Five operant chambers (20 × 15 cm) with curved rear walls (jointly developed with TSE, Bad Homburg, Germany) were used (Steckler et al., 2000). Set in the curved wall of each box was an array of five circular holes, 2 cm in diameter, 3 cm deep and 1.5 cm above floor level. Each hole was equipped with an infra-red photocell beam crossing the entrance vertically, a yellow LED at the rear end of the hole, providing 5 lux illumination at the hole's entrance, and a food pellet dispenser which allowed delivery of a 20 mg dustless food pellet (Noyes, Lancaster, UK) directly into the hole where a correct response had been made. Presentation of the light stimulus, the

operandum and the food reward all in the same location was chosen to enhance stimulus-response-reward contiguity. In addition, a white house light (30 lux) mounted in the centre of the roof could illuminate the chamber. The boxes were housed in dark, sound-attenuating compartments. On-line control of the apparatus and data collection was performed using a computer system with software written by TSE (OBS, Version 1.56).

5-CSRTT: Habituation

Mice were first exposed to the boxes for 20 min per session, with the house light switched off and each hole containing two food pellets. Once all animals reliably ate all food pellets within a session, training proceeded to the next stage.

5-CSRTT: Autoshaping

Autoshaping was carried out as described by Steckler et al. (2000). During autoshaping, all lights were switched off at the beginning of a session. A session terminated after a maximum of 20 completed trials or after 30 min, whichever came first. A trial started with the illumination of one of the five holes (in pseudorandom order), the house light being switched off. If an animal did not respond by nose poking into the illuminated hole within a limited hold of 5 s, a pellet was delivered into that hole. The stimulus light remained illuminated until a response into that hole (arbitrarily designated 'correct') had been made to collect the pellet, after which the stimulus light was switched off. If an animal responded into the illuminated (correct) hole within the limited hold of 5 s, a pellet was delivered immediately and the stimulus light was extinguished prior to the end of the limited hold. A response into the correct hole initiated an inter-trial interval (ITI) of 5 s, during which only the

house light was illuminated. This was followed by the next trial. Responses into the other holes were counted but had no further consequences.

5-CSRTT: Simple visual discrimination learning

The following sessions always started after a 2 min habituation period, during which all lights were switched off, and terminated after 60 trials or after 30 min, whichever came first. At the end of the 2 min habituation, the first trial commenced. Each trial started with the illumination of one of the holes (in pseudorandom order) for a maximum of 8 s or until a response was made. Animals had to respond within a limited hold of 10 s, i.e., a nose poke made within the 2 s after the 8 s-light stimulus had been switched off was still considered a response. Nose poking into the hole where the stimulus was presented was considered to be correct. This was followed by the delivery of a food pellet into that hole and the stimulus light was extinguished immediately if the response occurred within the first 8 s, followed by an inter trial interval (ITI) of at least 5 s, during which all lights were turned off. A response into another hole was recorded as an incorrect response, the stimulus light in the correct hole was extinguished, if still necessary, but no food pellet was given. If an animal did not respond within the limited hold of 10 s, this was counted as an omission. Incorrect responses or failures to respond (omission) resulted in a time out period of 5 s, during which only the house light was illuminated. After termination of the time out period, an ITI of at least 5 s duration commenced, followed by the next trial. In order to ensure that stimulus presentation was not overlapping with a hole poke, which could potentially prevent an animal from stimulus detection, the next trial only commenced if no holes were visited for at least 1 s prior to the end of the ITI, i.e., the ITI could continue over and above the 5 s limit. This was followed by gradual

reduction of the stimulus duration (i.e., 8 s, 4 s, 2 s, 1 s), with 5 sessions per stimulus duration.

5-CSRTT: Baseline performance

Subsequently, animals were tested for extended number of sessions with a stimulus duration of 0.5 s.

5-CSRTT: Measures

During autoshaping, the number of nose-pokes made until the illuminated hole was visited, the average latency to complete a trial, and the relative number of trials completed within the limited hold of 5 s, i.e., prior to pellet delivery [= number of trials completed within the limited hold / total number of trials completed per session $\times 100$], were studied.

The number of correct responses determined the absolute number of reinforcers earned. Accuracy was measured in terms of percentage correct responses [= correct responses / (correct + incorrect responses) $\times 100$]. Errors of omission equalled the number of trials during which no response was made within the limited hold of 10 s. Furthermore, the number of nose-pokes made during the ITI was counted and expressed per ITI, normalised to 5 s as ITI's could be longer, given that one second had to lapse between the last response made during the ITI and the beginning of the next trial. Moreover, correct and incorrect response latencies were measured, defined as the time between stimulus onset and the response. Finally, bias was evaluated by calculating the absolute number of correct responses made into the two left-hand holes minus the number of correct responses made into the two right-hand holes,

divided by the total number of correct responses made into these four holes. This measure has close resemblance to the bias measure Index Y, which has been suggested to reflect 'cognitive bias' (Sahgal, 1987). Unbiased responding would result in Index Y = 0. This index increases if an animal stays for a prolonged time at a particular position, responding in a go/no-go-like manner, and reflects regression towards a simpler problem solving strategy.

Plasma corticosterone and ACTH

Animals were anaesthetised with isofluran and decapitated within 15 seconds. Trunk blood was collected in ice-chilled EDTA-coated tubes containing 140 µg aprotinin (Trasylol; Bayer, Cologne, Germany). Plasma was separated and stored at -80°C until corticosterone or ACTH measurement. Plasma ACTH and corticosterone concentrations were measured using commercially available radioimmunoassay kits (ICN Biomedicals, Costa Mesa, USA). The inter- and intraassay coefficients of variability for ACTH were 7 and 5 % with a detection limit of 2 pg/ml. For corticosterone, the inter- and intraassay coefficients were 7 and 4 %, respectively, with a detection limit of 1.5 ng/ml.

Statistics

Data were analysed by multiple analyses of variance (MANOVA), followed by post hoc Tukey test if appropriate.

Chapter 3

Behavioural analysis of four mouse strains in an anxiety test battery

Introduction

Recent advances in molecular biology have led to the generation of an increasing number of genetically engineered mice with specific mutations in single genes. However, such mutations can have very different phenotypes, for example with respect to anxiety-related behaviour, depending on genetic background (Banbury Conference on Genetic Background in mice, 1997; Frankel, 1998). Indeed, strain differences in murine anxiety paradigms are well established (Crawley et al., 1997; Gerlai, 1996; Homanics et al., 1999; Rogers et al., 1999; Trullas and Skolnick, 1993) – differences which do not only affect the outcome of experiments with mouse mutants, but which are of course of equal relevance for pharmacological (Conti et al., 1994; Crawley and Davis, 1982, 1997; Lepicard et al., 2000) or lesion studies.

Here, three inbred mouse strains were compared in these tests: the A/J strain, which has been suggested to show a high degree of anxiety-related behaviour (Crawley et al., 1997; Crawley and Davis, 1982), the C57BL/6J strain, which has been suggested

to display intermediate levels of anxiety (Crawley and Davis, 1982) and which is commonly used for the generation of mouse mutants, and the C57BL/6ChR strain. The C57BL/6ChR subline originated from the C57BL/6J line, but both sublines have been bred independently for several generations since 1974 and are provided by different suppliers. Previous studies have shown genetic divergence in a number of inbred strains, and certain C57BL/6 sublines show marked differences in behaviour, for example in exploratory activity (Crusio et al., 1991). Finally, the Swiss Webster outbred strain was tested. This strain was chosen as a literature search revealed the Swiss Webster mouse to be a popular mouse strain for pharmacological tests of anxiety-related behaviour.

Anxiety-related behaviour was assessed using a battery of tasks, taxing different aspects of anxiety-related behaviour (see introduction), such as free exploration of novel environments, free exploration of a novel object, but based within a familiar surrounding, conflict behaviour and conditioned fear.

Material and Methods

Animals

Male A/J mice (n = 11), C57BL/6 J mice (B6J; n = 10) and Swiss Webster mice (SW/J; n = 11) were supplied by Harlan Winkelmann (Borchen, Germany), while C57BL/6CrIBR mice (B6/ChR; n = 10) were supplied by Charles River (Sulzfeld, Germany). Animals were singly housed in type II cages and kept under 12:12 h light-dark cycle as of two weeks before testing, with food and water *ad libitum*. All testing took place during the light phase of the light-dark cycle, except for the elevated plus

maze test, which was performed during the early dark phase. Animals were aged 8 – 9 weeks at the beginning of testing.

Behavioural analysis

After placing the animal in a confinement for 1 min, spontaneous behaviour was analysed in the home cage for ten minutes. Counting the number of line crossings was used as a measure to assess locomotor activity. Next, animals were tested on the elevated plus maze. Then, animals were tested in the light-dark exploration paradigm. Locomotor activity was further assessed in a 30 minutes open field session, after which the response to the introduction of a novel object in a familiar environment was investigated in the novel object exploration paradigm. Conflict behaviour was investigated in the Vogel conflict task. Further, exploratory behaviour was analysed in the hole board paradigm, after which conditioned fear was studied. Furthermore, pain threshold was assessed using the hot plate test, and 24-hour water intake was measured. Finally, animals were exposed to inescapable swim stress, of which the immobility time during the last three min of testing was scored. At least 5 days lapsed between tests.

Results

Examination of spontaneous behaviour

Locomotor activity in the home cage, as measured by the number of line crossings, was significantly different between strains ($F_{1,38} = 67.05$, $p < 0.001$; Figure 3.1A). Post hoc analysis showed the following rank order: SW/J > B6/J = B6/ChR > A/J. Furthermore, locomotor activity decreased over time in all strains ($F_{1,38} = 334.65$, $p <$

0.001). Analysis of rearing activity also revealed an effect of strain ($F_{3,38} = 86.66$, $p < 0.001$), with the SW/J mice spending more time rearing than the other three groups, and the B6/ChR mice rearing more than mice of the A/J strain (Figure 3.1B). Strains also differed in the time spent grooming ($F_{3,38} = 14.80$, $p < 0.001$), whereby A/J mice groomed significantly more than animals from the other strains (Figure 3.1C). Finally, there was an effect of strain on the time spent digging in the sawdust ($F_{3,38} = 22.01$, $p < 0.001$), with the following rank order: B6/J > B6/ChR > SW/J = A/J (Figure 3.1D).

Elevated plus maze

The time spent on the central platform was significantly different between strains ($F_{3,36} = 11.62$, $p < 0.001$), with the A/J strain spending more time on the central platform compared to all other groups (Figure 3.2A). A strain difference was also seen in terms of total arm entries ($F_{3,36} = 45.07$, $p < 0.001$), and post hoc comparison indicated that the SW/J mice were more active than all the other strains (Figure 3.2B). Latency to enter the open arm also differed between strains ($F_{3,36} = 3.46$, $p = 0.026$). Post hoc analysis showed that A/J mice had longer latencies than B6/J animals (Figure 3.2C). Moreover, strains differed in the total time spent on the open arms ($F_{3,36} = 8.74$, $p < 0.001$), with B6/J mice spending significantly more time there than the SW/J or the A/J strains (Figure 3.2D). When the relative number of entries into the open arms was analysed, MANOVA revealed a further effect of strain ($F_{3,35} = 3.49$, $p = 0.026$), but post hoc testing failed to show significant group differences (Figure 3.2E).

Spontaneous Behaviour

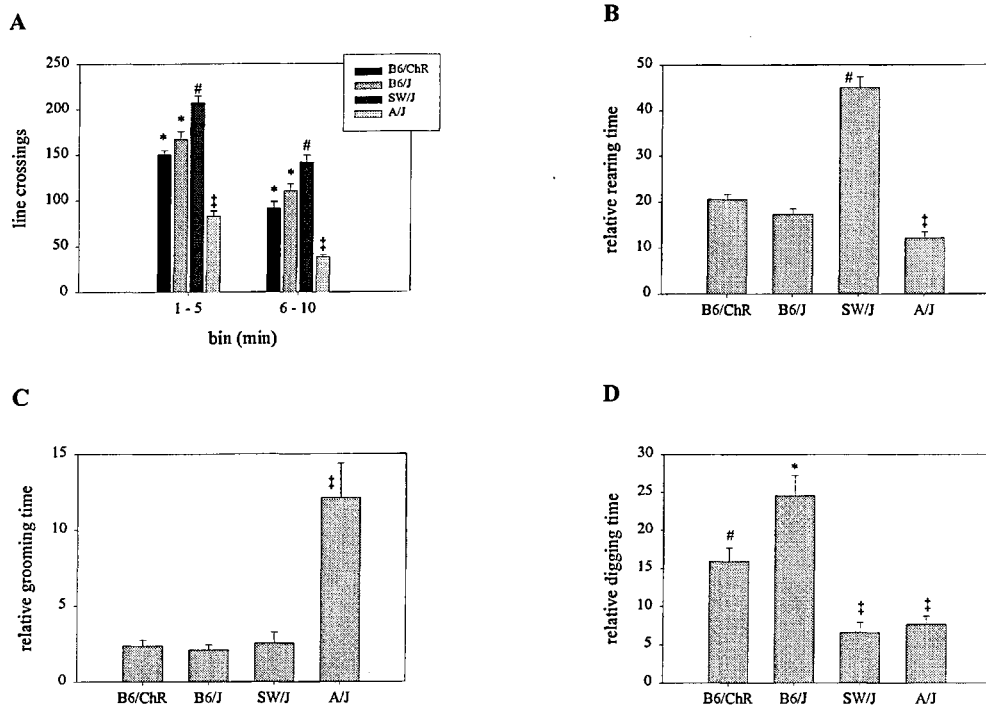


Figure 3.1 Strain dependent differences in spontaneous behavioural in the home cage. Line crossings (A) were quantified over two successive 5-min time bins (means with different symbols indicate $p < 0.05$). The times spent rearing (B; # $p < 0.05$ compared to all other groups; ‡ $p < 0.05$ compared to B6/ChR), grooming (C; ‡ $p < 0.05$ compared to all groups) and digging (D; means with different symbols indicate $p < 0.05$) were analysed over 10 min. Data are presented as means, with error bars denoting SEMs.

Light dark exploration

There was a strain effect in terms of the total distance travelled ($F_{3,38} = 22.81$, $p < 0.001$). A/J animals were less active than all the other strains (Figure 3.3A). The total number of rearings was also significantly different between strains ($F_{3,38} = 4.61$, $p = 0.008$), and A/J mice reared less frequently than the two B6 strains (Figure 3.3B). Furthermore, strains differed in the number of entries into the light ($F_{3,38} = 2.89$, $p = 0.048$), which were increased in the B6/J animals compared to B6/ChR mice, in

Elevated Plus Maze

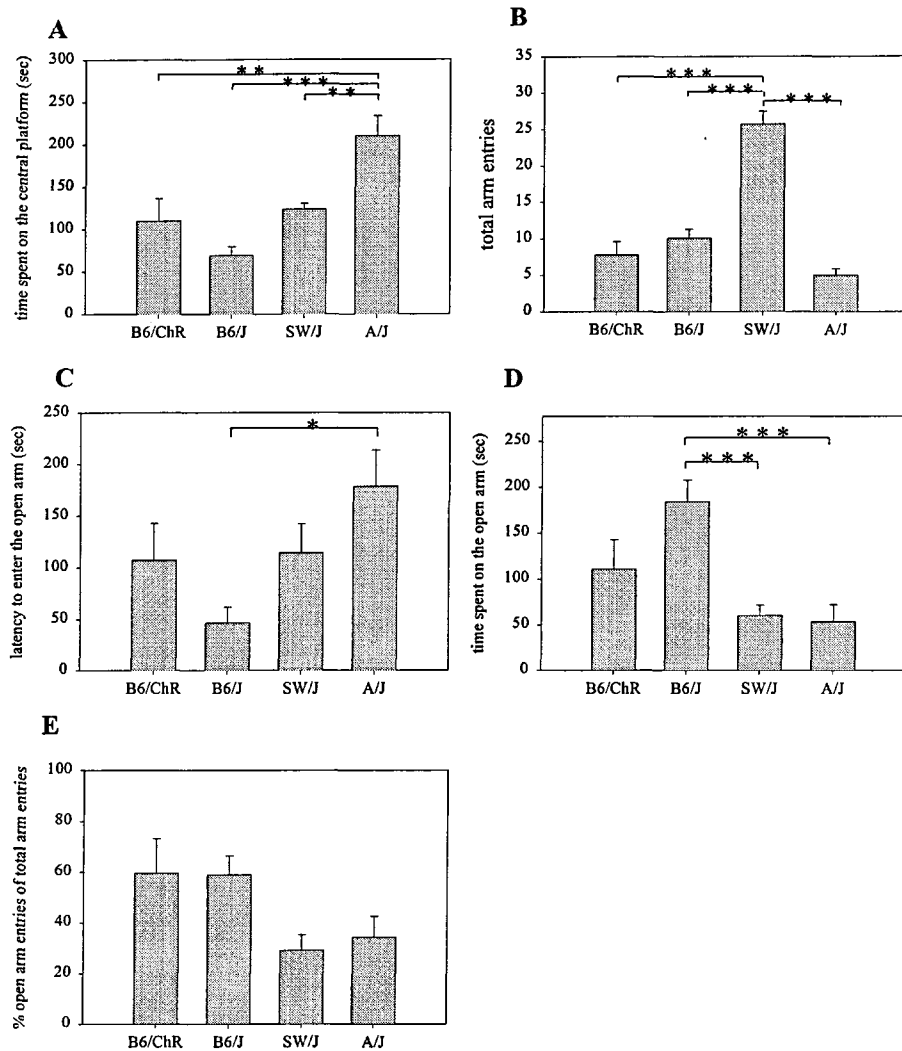


Figure 3.2 Strain dependent differences in elevated plus maze exploration: the time spent on the central platform (A), total arm entries (B), the latency to enter an open arm (C), the time spent on the open arms (D) and the relative number of open arm entries (E) are shown. Data are presented as means, with error bars denoting SEMs; *, $p < 0.050$; **, $p < 0.010$; ***, $p < 0.001$, significant differences between strains.

Light -Dark Box

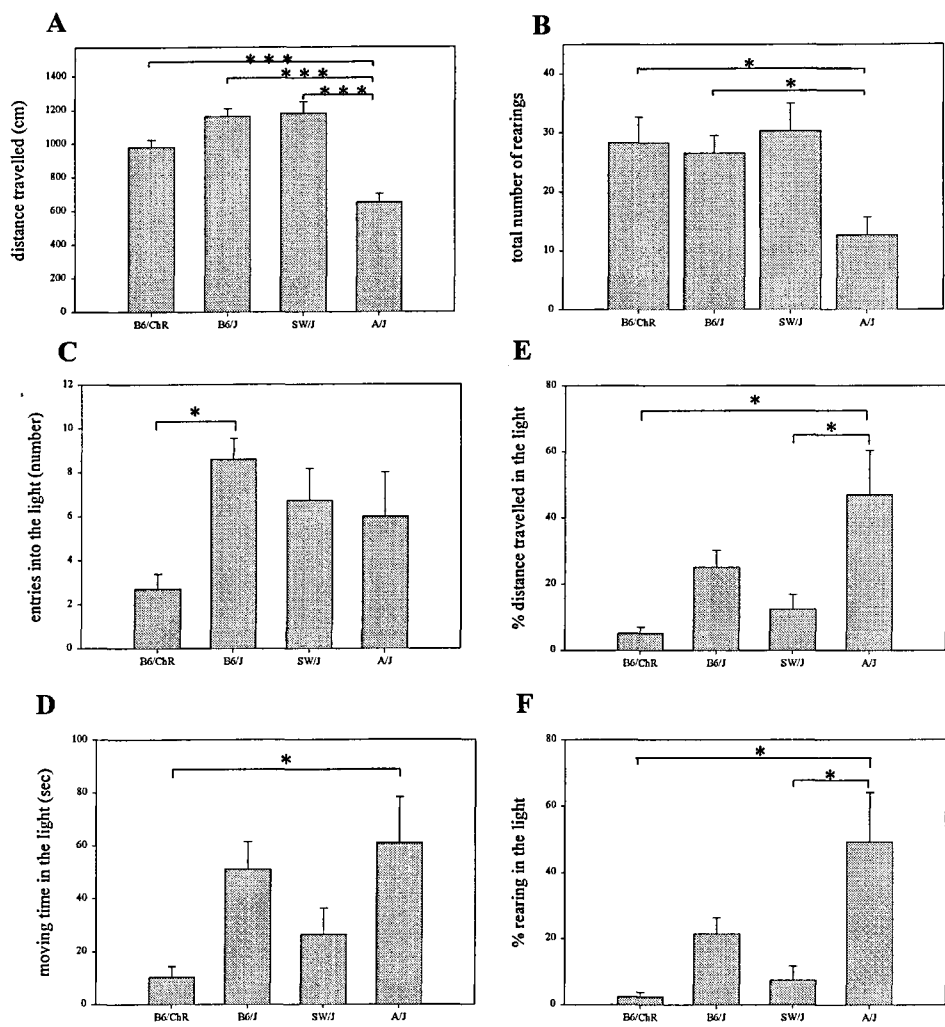


Figure 3.3 Strain dependent differences in light dark box exploration: the total distance travelled over 5 min (A), the total number of rearings (B), the number of entries into the illuminated part of the box (C), the time the animal spent moving in the illuminated part (D), the relative distance travelled in the light (E) and the relative number of rearings made in the illuminated area (F) are shown. Data are presented as means, with error bars denoting SEMs; *,p < 0.050; ***p < 0.001, significant differences between strains.

terms of moving time in the light ($F_{3,38} = 3.83$, $p = 0.017$), in the relative distance travelled ($F_{3,38} = 5.44$, $p = 0.003$), and in the relative number of rearings made in the illuminated part of the box ($F_{3,38} = 6.35$, $p < 0.001$) (Figures 3.3C-F). Post hoc analyses revealed that A/J animals made relatively more rearings and travelled a relatively greater distance in the illuminated half of the box than both B6/ChR and SW/J mice, and spent more time moving in the illuminated part than B6/ChR animals.

Open field

A significant effect of time bin was found on the distance travelled measure during the 30 min habituation stage ($F_{9,342} = 4.22$, $p < 0.001$; Figure 3.4). Furthermore, an overall significant strain difference ($F_{3,38} = 26.85$, $p < 0.001$), but no strain \times bin interaction ($F_{27,342} = 1.29$, $p > 0.050$) was seen for this measure. Post hoc analysis indicated that the A/J mice were hypoactive compared to all other strains, and that SW/J animals showed significant higher locomotor activity than B6/ChR mice did. No preference for a particular quadrant of the field was observed during the habituation stage according to time spent in the different quadrants ($F_{3,152} = 2.60$, $P > 0.050$).

Open Field Habitation

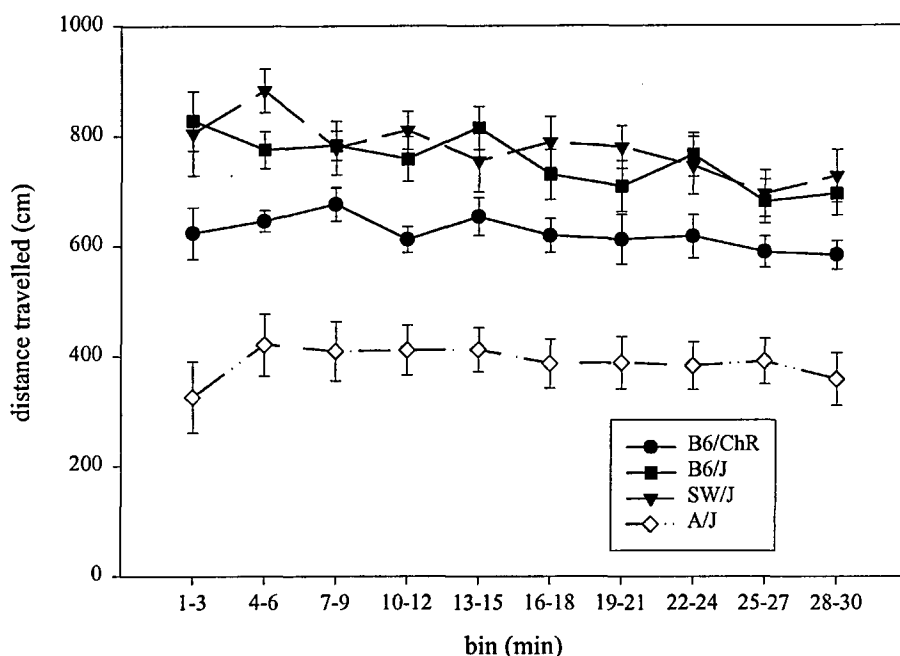


Figure 3.4 Strain dependent differences in habituation to the open field prior to presentation of a novel object in terms of the distance travelled. Data are presented as means, with error bars denoting SEMs.

Novel object exploration

After introduction of the novel object in the open field, strains differed in the total time spent in the object quadrant ($F_{3,38} = 6.48$, $p = 0.001$), and a significant genotype \times bin interaction was observed ($F_{12,152} = 2.56$, $p = 0.010$). This interaction was due to the fact that SW/J mice spent more time in the object quadrant compared to all other groups during the first three minutes of exposure to the object (Figure 3.5A), and continued to differ significantly during the second 3-min bin from both the B6/J and

A/J strains (data not shown). A strain effect was also seen if the time spent in the object quadrant relative to baseline was calculated ($F_{3,38} = 3.21$, $p = 0.034$), and a significant strain \times bin interaction was found for this measure ($F_{12,152} = 2.25$, $p = 0.012$). As for the quadrant preference measure, SW/J mice spent more time in the object quadrant than all other groups if the relative change from baseline was considered (Figure 3.5B). In addition, analysis of the change from baseline also revealed that B6/ChR animals spent more time in the object quadrant than A/J mice during the first 3 min of object exposure did. Strains did not differ during subsequent bins on this measure.

Visual inspection of the data indicated that the strains reacted very differently to the introduction of the novel object, varying from a avoidance, most pronounced in the A/J strain, to approach of the object quadrant, which was strongest in the SW/J strain, while the two B6 strains seemed to react rather indifferently (Figure 3.5B).

Analysis of the number of entries into the object quadrant relative to baseline also showed an effect of strain ($F_{3,38} = 4.17$, $p = 0.012$), and a strain \times bin interaction was observed ($F_{12,152} = 2.24$, $p = 0.022$). As before, A/J mice showed avoidance of the object during the initial three minutes, while a relative increase of entries into the object quadrant was seen in the B6/ChR strain, and both the B6/ChR and the SW/J strains differed from A/J mice during the first two time bins (Figure 3.5C).

Novel object exploration

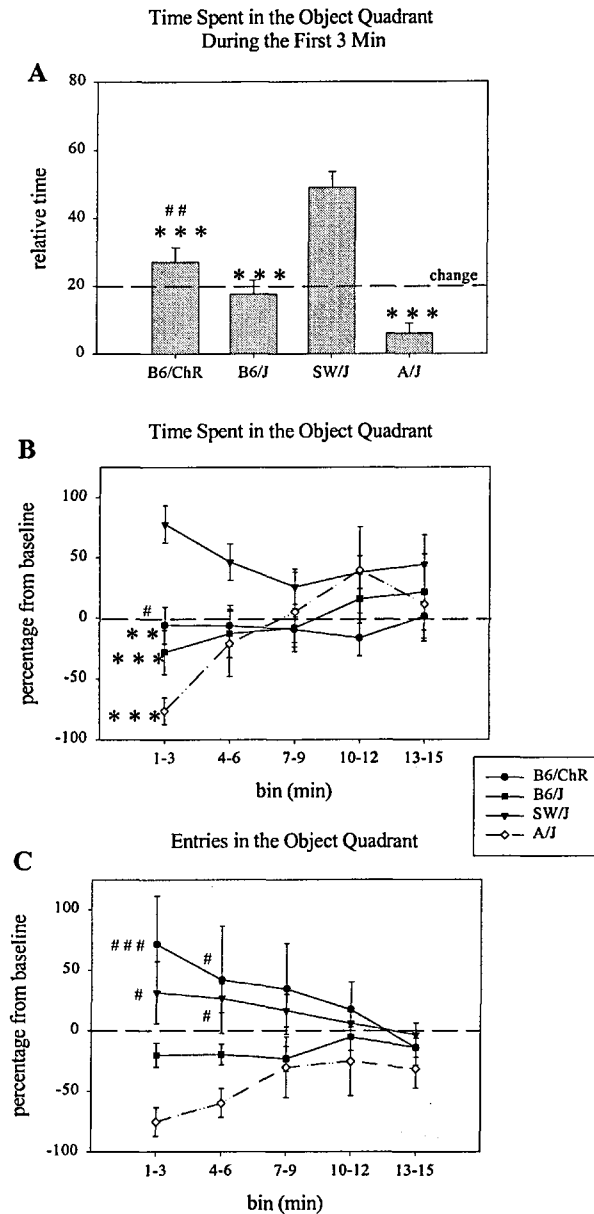


Figure 3.5 Strain dependent differences in novel object exploration: strains differed in quadrant preferences during the first 3-min time bin following exposure to the object (A), and in the relative change from baseline in terms of the time spent in the object quadrant (B) and the number of entries made into that quadrant (C). The dotted line in (A) represents chance performance, while the dotted lines in (B) and (C) indicate baseline levels. Data are presented as means, with error bars denoting SEMs; **, $p < 0.010$; ***, $p < 0.001$, significant difference compared to SW/J mice; #, $p < 0.050$; ###, $p < 0.001$, significant difference compared to A/J.

Vogel conflict task

There was an effect of strain on the number of shocks delivered during the conflict session ($F_{1,28} = 3.50$, $p = 0.028$; Figure 3.6A). SW/J mice received significantly more shocks than A/J mice did. No significant difference was found for the latency to start the session measure ($F_{1,28} = 0.95$, $p = 0.432$; Figure 3.6B).

Hole board exploration

Strains differed in the distance travelled on the hole board ($F_{3,41} = 31.90$, $p < 0.001$). Again, A/J animals were hypoactive compared to the other three strains (Figure 3.7A). Likewise, an effect of strain was found in the number of rearings ($F_{3,41} = 10.15$, $p < 0.001$), with the A/J strain being the least active (Figure 3.7B). Analysis of the total number of nose pokes also revealed an effect of strain ($F_{3,41} = 16.26$, $p < 0.001$), with SW/J mice visiting more holes than the other strains. A/J mice did not differ from the two B6 strains on this measure (Figure 3.7C).

Vogel Conflict Task

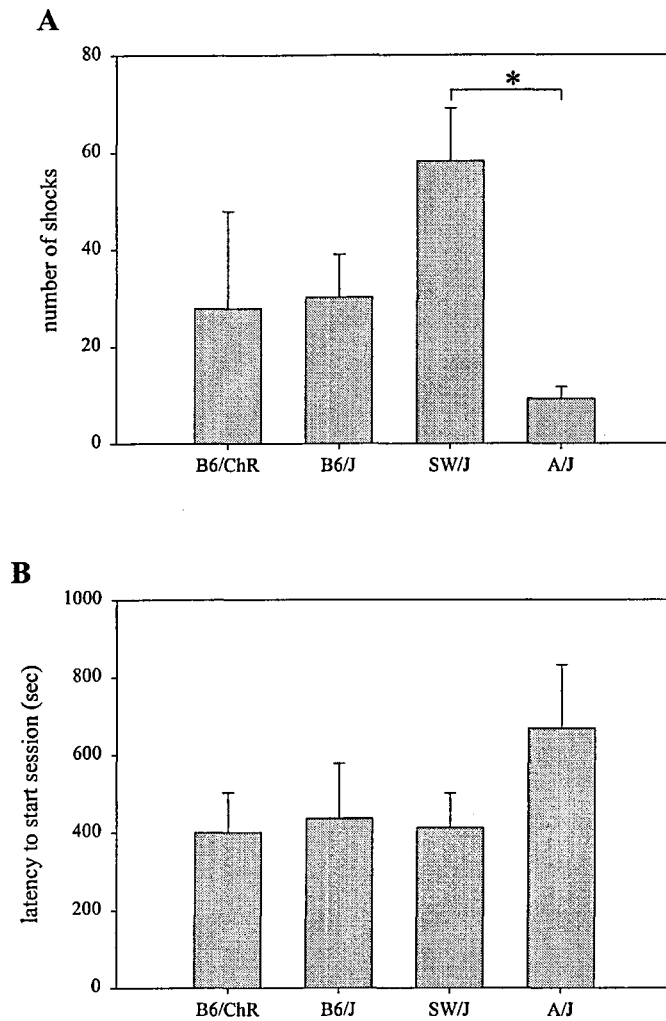


Figure 3.6 Strain dependent differences in conflict behaviour: number of shocks received (A) and latency to start the session (B). Data are presented as means, with error bars denoting SEMs; *, $p < 0.050$, significant difference between SW/J and A/J.

Conditioned fear

When the relative time not moving during re-exposure to the CS was analysed, MANOVA showed a strain \times minute interaction ($F_{12,132} = 4.89$, $p < 0.001$). Further analysis revealed that all strains displayed a comparable rise in the time not moving following presentation to the CS (approximately 25% increase from baseline; Figure 3.8). However, strains showed different degrees of extinction to the stimulus. Thus, activity in the two B6 strains started to return to baseline after the first minute, while extinction was retarded in the SW/J strain compared to both B6 strains. Although SW/J mice again reached baseline at the end of the session, they spent significantly less time moving than B6/J mice during the second, third and fourth minutes, and also differed from B6/ChR mice during minute three. In contrast, A/J mice did not return to baseline and differed from the two B6 strains from the third minute onwards. No significant differences were found between the two B6 strains or between A/J and SW/J strains.

Analysis of the change in the distance travelled relative to baseline also revealed a strain \times time interaction ($F_{12,132} = 6.35$, $p < 0.001$). Post hoc testing showed a pattern similar to the one described for the time not moving measure (data not shown).

16 Hole Board

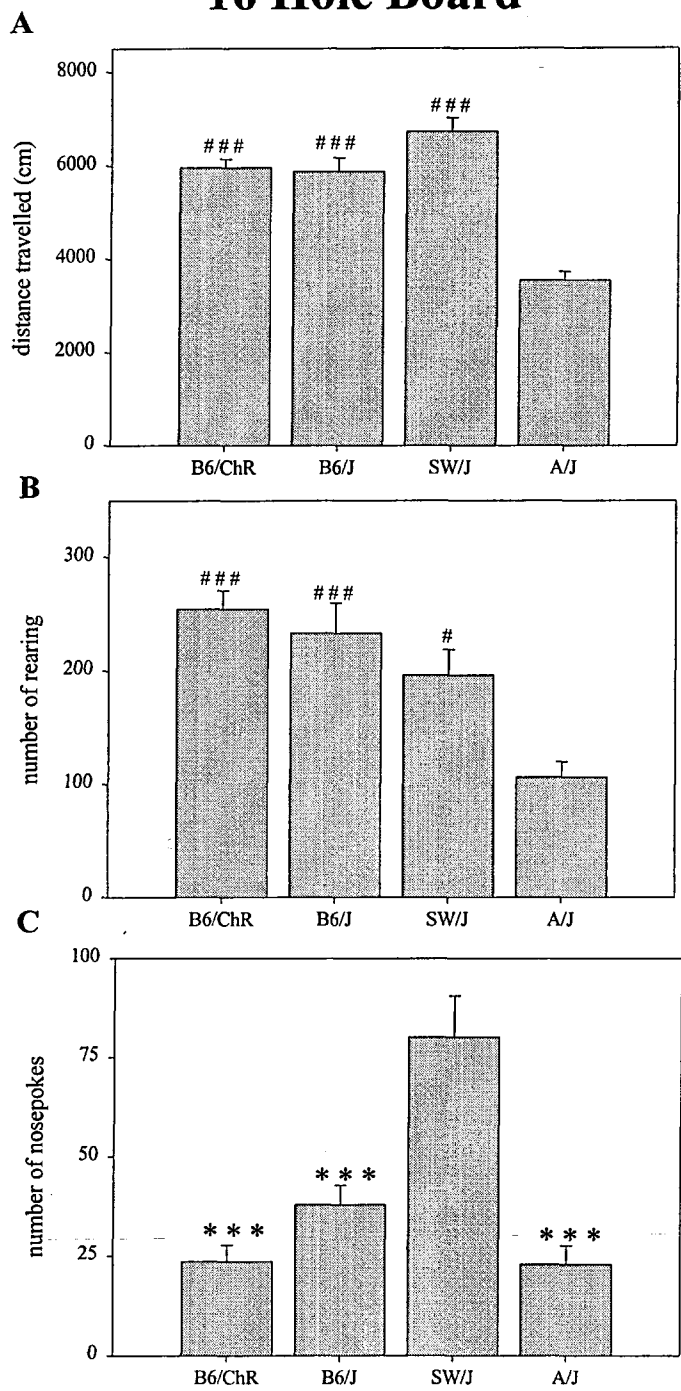


Figure 3.7 Strain dependent differences in hole board exploration: distance travelled (A), number of rearings (B) and number of nose pokes (C) made during a 30 min session are shown. Data are presented as means, with error bars denoting SEMs; ***, $p < 0.001$, significant difference compared to SW/J; #, $p < 0.050$; ###, $p < 0.001$, significant difference compared to A/J.

Conditioned Fear

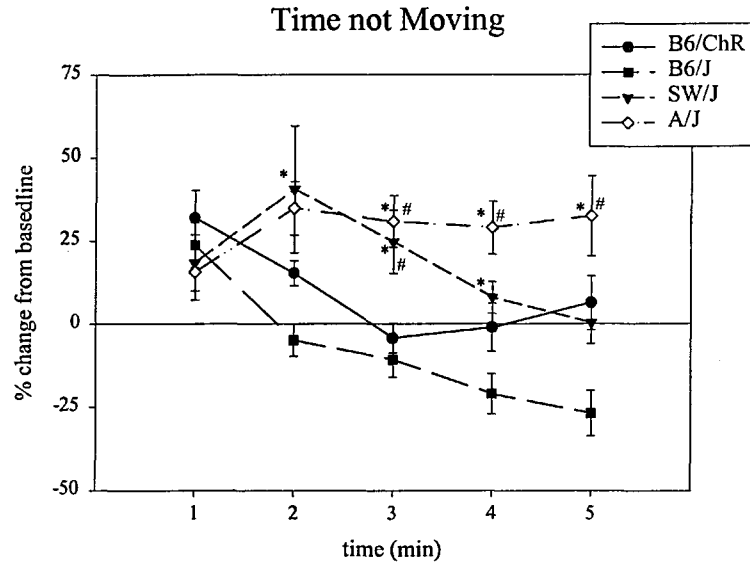


Figure 3.8 Strain dependent differences in conditioned fear: change in the time not moving during re-exposure to the light relative to baseline (dotted line). Data are presented as means, with error bars denoting SEMs, * $p < 0.05$ compared to B6/J, # $p < 0.05$ compared to B6/ChR.

Hot plate

During the hot plate test, no jumping was observed. Latencies to lick the feet were significantly different between strains ($F_{3,38} = 10.61$, $p < 0.001$; Table 3.1), and a lower pain threshold was seen in SW/J mice compared to the other strains.

Inescapable Swim Stress

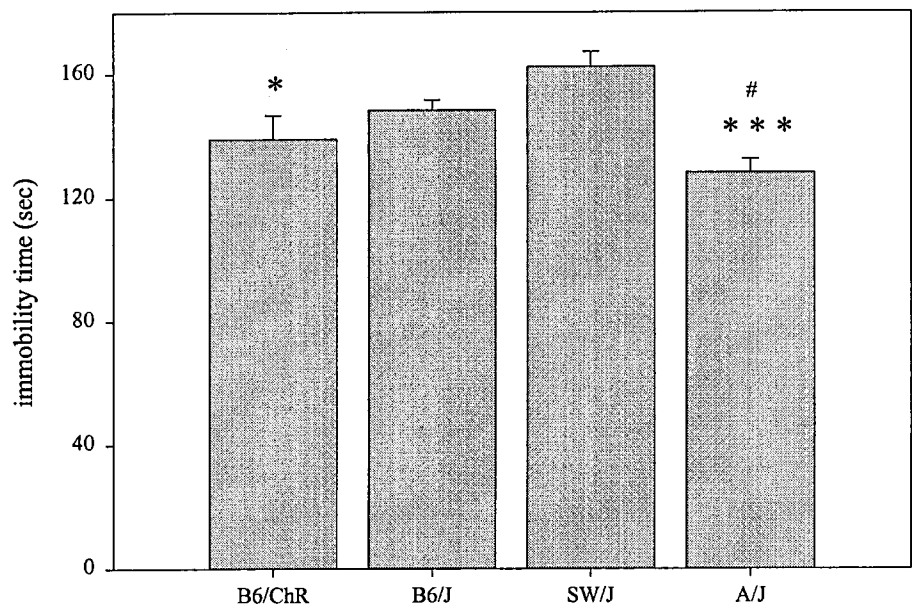


Figure 3.9 Strain dependent difference in immobility time during inescapable swimming: immobility time during the last three minutes of testing. Data are presented as means, with error bars denoting SEMs; *, $p < 0.050$; ***, $p < 0.001$, significant difference compared to SW/J, #, $p < 0.050$, significant difference compared to B6/J.

Table 3.1: Water intake and pain threshold in four mouse strains.

	B6/ChR	B6/J	SW/J	A/J
water intake (ml)	3.5 ± 0.1	4.0 ± 0.3	5.4 ± 0.5 *	3.5 ± 0.2
hot plate latency (sec)	8.5 ± 0.8	8.6 ± 0.5	4.6 ± 0.5 *	7.6 ± 0.5

Data are presented as means, with error bars denoting SEMs; *, $p < 0.050$, significant different to the other strains.

Inescapable Swim stress

An effect of strain was also seen for immobility time ($F_{1,37} = 7.90$, $p < 0.001$), and SW/J mice spent more time immobile than B6/ChR and A/J animals. Furthermore, immobility time was increased in B6/J mice relative to immobility in the A/J strain (Figure 3.9).

Discussion

In general, SW/J mice were the most active strain in terms of locomotor activity (distance travelled), while locomotor activity was intermediate in the two B6 strains. A/J mice were the least active. A comparable rank order was found when the numbers of rearing were compared in the different paradigms. Although clear strain-dependent effects were also seen in measures of anxiety-related behaviour, the rank order of strain effects was more variable and task-dependent, which suggests that the different tasks may measure different aspects of anxiety-related behaviour independent of differences on locomotor activity.

Differences between the two B6 sublines were absent with two exceptions: B6/J mice spent more time burying in the home cage and made more entries into the illuminated part of the light dark box than B6/ChR mice, but did not differ significantly from each other in any other measure. This suggests that both sublines display comparable level of anxiety-related behaviour.

B6 mice of both lines were the least anxious on the elevated plus maze. No differences were found between the SW/J and the A/J mice in terms of open arm exploration. The A/J mice, however, spent more time on the central platform than the three other strains. These results are comparable to those reported by others comparing B6/J and A/J mice on this task (Trullas and Skolnick, 1993). The two B6 strains also displayed relatively low levels of anxiety-related behaviour in the fear conditioning paradigm, while A/J mice showed strongest responses. Likewise, A/J mice spent more time grooming in the home cage, spent less time in the object quadrant than the other strains during exploration of a novel object – in fact seemed to avoid this quadrant – and had the lowest number of licks in the Vogel conflict paradigm. These results support earlier findings that A/J mice display high level of anxiety-related behaviour when compared with other mouse strains (Brodin et al., 1998; Crawley et al., 1997; Crawley and Davis, 1982). Although it could be argued that A/J mice showed reduced anxiety-related behaviour in the light dark box in terms of the relative activity in the illuminated compartment, which would contrast earlier reports of enhanced anxiety-related behaviour of A/J mice on this task (Crawley and Davis, 1982), it should be noted that locomotor activity of A/J mice was very low in the present study. Thus, starting the mice in the illuminated part of the box may have skewed the data towards a “preference” for the illuminated area.

According to time spent in the object quadrant during novel object exploration, a rank order of SW/J < B6/ChR = B6/J < A/J was found. A similar rank order was seen for the number of licks in the Vogel paradigm.

Since responses in the Vogel conflict and conditioned fear paradigms are potentially confounded by differences in pain threshold, nociception was tested on the hot plate. These results suggest that the SW/J mice have a reduced pain threshold compared to the other strains. This in turn indicates that the increase in the time not moving in the conditioned fear paradigm seen in SW/J mice could be confounded by altered nociception. However, this explanation could not account for the performance in the Vogel conflict task, where SW/J mice received most shocks. Motivational factors, however, could have affected the number of licks in SW/J mice in this task, as enhanced water intake was seen in this strain. Thus, the two tasks based on punishment would not allow firm conclusions about anxiety levels of the SW/J strain. However, SW/J showed higher explorative activity on the hole board, while general activity did not differ from that of B6 mice, which would again suggest that SW/J mice are less anxious than B6 animals.

Interestingly, the analysis of immobility time in the swimming paradigm also showed a rank order of SW/J>B6>A/J. Immobility is unlikely to simply reflect locomotor activity, as a reversed rank order would have been expected. Immobility time has been suggested to be a useful predictor for antidepressant activity (Porsolt et al., 1977). However, many antidepressant drugs are also potent anxiolytic substances (Uhlenhuth et al., 1999), and immobility has also been reported to increase after treatment with benzodiazepines such as diazepam or flurazepam at doses which were not muscle relaxant (Nagatani et al., 1987). Moreover, struggling in the swimming paradigm has been reported to be negatively correlated with anxiety-related behaviour (Ferre et al., 1994). Thus, it is possible that immobility time in this task also depends on the degree of anxiety-related behaviour.

It could be argued that repeated testing of the animals might have affected performance. However, Mc Ilwain and colleagues (2001) have shown that test battery mice and naive mice show similar levels of anxiety-related responses in an open field test, light dark exploration task but also in conditioned fear response. Furthermore, the authors state that a test battery approach is the best strategy for obtaining the most amount of information using the fewest number of mice, which is especially important when subjects are difficult to obtain which is the case when mutants are being used.

In summary, the data indicated that locomotor activity was independent of the paradigm employed, while the rank order of strain-dependent effects on anxiety-related behaviour changed as a function of the task under study. This indicates that different anxiety paradigms tax different aspects of anxiety, suggesting that a battery of different tests is beneficial in studies of anxiety-related behaviour.

Chapter 4

Task-dependent effects of chronic citalopram on treatment on murine anxiety-related behaviour

Introduction

SSRI's have become a standard treatment for depression and anxiety disorders over the past years. Efficacy has been demonstrated for obsessive compulsive disorder, panic disorder and social phobia (Goodman et al., 1989; Lecrubier et al., 1997; Stein et al., 1998; Nutt et al., 1999). Results from small studies also suggest efficacy in posttraumatic stress disorder and generalised anxiety disorder (van der Kolk et al., 1994; Rocca et al., 1997; Marshall et al., 1998).

Preclinical data, however, are conflicting, varying from anxiolytic to anxiogenic effects after SSRI treatment (for review see: Griebel, 1995). This variability is probably due to a number of factors, including administration route (Treit, 1991), dose range (Soderpalm et al., 1989), species differences (Barrett and Gleeson, 1991), gender effects (Hughes, 1993), environmental effects (Wettstein, 1992) and level of stress exposure before testing (Keeney and Hogg, 1999; Berton et al., 1999). However, different effects in different anxiety tasks may also be seen because different anxiety paradigms assess different aspects of anxiety. In order to investigate

whether the effects of SSRI's are task specific, we treated mice with citalopram, which is the one of the most selective SSRI's, and investigated anxiety-related behaviour in a murine anxiety test battery. With this protocol, results obtained from the different paradigms can be compared. Since clinical evidence indicates that the anxiolytic effects of SSRI's generally occur after long-term treatment and that anxiogenic effects can be seen early in the course of treatment, we tested the effects of chronic administration of citalopram on anxiety-related behaviour in mice. From the results from the previous study, it was decided to use Swiss Webster mice as test subjects.

Material and Methods

Animals

Male Swiss Webster mice (n = 10-11 per group; 8 – 9 weeks of age) were supplied by Harlan Winkelmann (Borchen, Germany).

Treatment

Animals were treated chronically with citalopram (racemic, kindly donated by Lundbeck, Copenhagen, Denmark) at a dose of 0, 10 and 20 mg/kg po. The drug was administered orally in pellet form derived from drying a mixture of powdered mouse food (Altromin 1311, Altromin GmbH, Lage, Germany), citalopram and water, which was stored for a maximum of four days at 5° C. Animals received 1 g pellets per day between 17.00 and 17.30 h, placed directly into the home cage, in addition to their normal food (access to normal food between 8.30 – 17.00 h). Pilot experiments had shown that mice show a preference for these pellets and tend to consume

immediately after delivery. Furthermore, no pellets were found before testing, indicating that all was consumed.

Behavioural analysis

After being treated for three weeks with citalopram, animals were tested on the elevated plus maze. Next, animals were tested in the light dark exploration paradigm. Locomotor activity was assessed in a 30 minutes open field session, after which the response to the introduction of a novel object in a familiar environment was investigated in the novel object exploration paradigm. Conflict behaviour was investigated in the Vogel conflict task, after which exploratory behaviour was assessed in the hole board paradigm. Furthermore, animals were tested in the conditioned fear paradigm. Next, pain threshold was assessed using the hot plate test. Finally, animals were exposed to inescapable swim stress, of which the immobility time during the last three min of testing was scored. At least 5 days lapsed between tests.

The animals were weighed before (day 1) and after 31 days of chronic treatment. Percentage body weight relative to day 1 was calculated.

Results

Elevated plus maze

Locomotor activity was not affected by treatment in terms of total number of arm entries ($F_{2,29} = 1.457$, $p > 0.05$; Figure 4.1A) or of the number of closed arm entries ($F_{2,29} = 0.168$, $p > 0.05$; data not shown). Open arm entries, however, were reduced

by chronic treatment with citalopram ($F_{2,29} = 3.491$, $p = 0.044$; Figure 4.1B), although post hoc testing did not indicate significant differences between groups. Furthermore, the time spent on the central platform was significantly increased by treatment ($F_{2,29} = 3.956$, $p = 0.030$; Figure 4.1C). Post hoc analysis revealed that animals treated with 20 mg/kg citalopram spent significantly more time on the central platform compared to vehicle treated mice ($p < 0.05$).

Light dark exploration

Chronic treatment with citalopram reduced locomotor activity as measured by the total distance travelled ($F_{2,29} = 3.696$, $p = 0.037$; Table 4.1). Post hoc analysis indicated that the total distance travelled was significantly reduced in animals treated with 20 mg/kg citalopram compared to controls ($p < 0.05$). Rearing was not affected by treatment ($F_{2,29} = 1.059$, $p > 0.05$; Table 4.1). No effect of treatment was found in terms of time spent in the light compartment ($F_{2,29} = 0.758$, $p > 0.05$; Table 4.1). Moreover, citalopram failed to affect the relative distance travelled and relative number of rearings made in the light ($F_{2,29} = 0.956$, $p > 0.05$ and $F_{2,29} = 1.168$, $p > 0.05$, respectively; Table 4.1).

Novel Object Exploration

Citalopram failed to significantly affect the distance travelled during the 30 min habituation session ($F_{2,29} = 1.804$, $p > 0.05$; Table 4.1), although a tendency similar to the effects on locomotor activity observed in the L/D box was seen. The number of rearings made were also not significantly affected ($F_{2,29} = 2.665$, $p > 0.05$; Table 4.1). Furthermore, the relative distance travelled in the centre and the time spent in the centre during the first 5 min remained unaffected by treatment ($F_{2,29} = 1.598$, $p > 0.05$ and $F_{2,29} = 1.216$, $p > 0.05$, respectively; Table 4.1).

Elevated plus maze

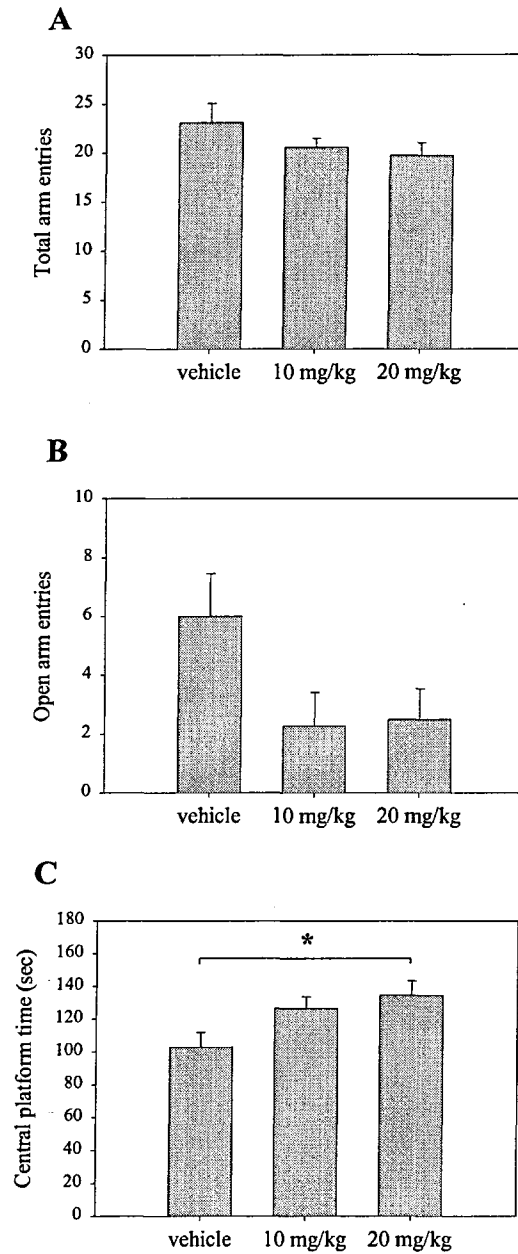


Figure 4.1 Effects of chronic citalopram treatment on elevated plus maze exploration: total arm entries (A), entries into the open arms (B) and time spent on the central platform (C) are shown. Data are presented as means, with error bars denoting SEMs; *, $p < 0.05$ significant differences between groups.

After the habituation session, the novel object was introduced. As presented in Table 4.1, the relative time spent in the object quadrant was above change level during the early testing phase, after which it returned to change level. However, no significant treatment x bin interaction ($F_{4,54} = 0.360$, $p > 0.05$) or overall difference between groups ($F_{2,27} = 0.435$, $p > 0.05$) were found for this parameter. Moreover, no significant treatment x time interaction ($F_{4,54} = 0.911$, $p > 0.05$, Table 4.1) or overall group difference ($F_{2,27} = 1.314$, $p > 0.05$) were found when relative entries in the object quadrant (Table 4.1) were considered.

Hole board exploration

Total distance travelled (Figure 4.2A) did not significantly differ between groups ($F_{2,29} = 1.583$, $p > 0.05$). Moreover, the number of rearings (Figure 4.2B) were not significantly altered by treatment ($F_{2,29} = 3.149$, $p > 0.05$). However, the number of nose pokes (Figure 4.2C) was dose-dependently increased by treatment ($F_{2,29} = 4.814$, $p = 0.016$). Post hoc testing revealed that animals treated with 20 mg/kg citalopram made more nose pokes compared to vehicle treated animals ($p < 0.05$).

Vogel Conflict Task

Conflict behaviour was not significantly altered by treatment, neither in terms of number of shocks received ($F_{2,24} = 0.046$, $p > 0.05$; Table 4.1), nor in latency to start the session ($F_{2,24} = 1.421$, $p > 0.05$; Table 4.1).

Table 4.1 Effects of chronic citalopram treatment on behaviour in the light dark exploration paradigm, open field, novel object exploration task, Vogel conflict paradigm and pain threshold. Data are presented as means \pm SEMs; *, $p < 0.05$ significant difference compared to vehicle treated animals.

Paradigm	Parameter	Vehicle	10 mg/kg	20 mg/kg
LD-Exp P	Distance travelled (cm)	1402 \pm 83	1246 \pm 53	1163 \pm 40 *
LD- Exp P	Number of rearings	49.0 \pm 7.4	42.7 \pm 4.9	37.3 \pm 3.5
LD- Exp P	Time in light (sec)	121 \pm 16	106 \pm 17	91 \pm 18
LD- Exp P	Rel. distance in the light	35.6 \pm 4.3	31.2 \pm 5.0	26.3 \pm 4.8
LD- Exp P	Rel. number of rearings in the light	38.5 \pm 5.9	31.3 \pm 6.3	25.1 \pm 6.3
Open field	Distance travelled (cm)	9368 \pm 1015	8310 \pm 443	7510 \pm 366
Open field	Number of rearings	276 \pm 28	254 \pm 16	204 \pm 21
Open field	Rel. distance travelled in the centre	17.4 \pm 1.5	18.6 \pm 1.1	14.4 \pm 1.6
Open field	Time spent in the centre (sec)	242 \pm 21	260 \pm 18	194 \pm 24
NOE	Rel. time in object quadrant min 1-3	34.9 \pm 5.3	40.2 \pm 2.9	40.3 \pm 5.6
NOE	Rel time in object quadrant min 4-6	33.9 \pm 3.8	35.3 \pm 2.2	33.0 \pm 3.5
NOE	Rel. time in object quadrant min 7-9	24.6 \pm 4.3	29.9 \pm 3.2	28.6 \pm 4.3
NOE	Rel. entries in object quadrant min 1-3	27.0 \pm 1.7	29.1 \pm 1.9	27.7 \pm 1.9
NOE	Rel. entries in object quadrant min 4-6	29.8 \pm 1.2	30.4 \pm 1.3	31.8 \pm 1.6
NOE	Rel. entries in object quadrant min 7-9	21.4 \pm 3.5	27.2 \pm 1.5	27.6 \pm 2.8
Vogel conflict	Number of shocks	34.1 \pm 14	36.9 \pm 10	40.2 \pm 17
Vogel conflict	Latency to start the session (sec)	347 \pm 78	215 \pm 34	255 \pm 56
Hot plate	Latency (sec)	8.7 \pm 0.9	9.6 \pm 0.9	8.5 \pm 1.1

LD-Exp P: light dark exploration paradigm, Rel: relative, NOE: novel object exploration.

Conditioned fear

The response during re-exposure to the CS was analysed by studying the relative time not moving and distance travelled, both calculated relative to baseline (Figure 4.3). No treatment x bin interaction was seen when the relative time not moving was considered ($F_{8,116} = 0.911$, $p > 0.05$), but overall, this measure tended to be reduced by treatment, just failing to reach significance ($F_{2,29} = 3.14$, $p = 0.058$). Furthermore, ANOVA revealed an overall effect on the relative distance travelled ($F_{2,29} = 4.223$, $p = 0.025$), but no treatment x bin interaction was seen ($F_{8,116} = 1.573$, $p > 0.05$). Post hoc testing revealed that animals treated with 20 mg/kg citalopram showed reduced relative distance travelled compared to vehicle treated animals ($p < 0.05$).

Hot plate

During the hot plate test, no jumping was observed. Latency to lick the feet (Table 4.1) was not significantly affected by chronic treatment ($F_{2,28} = 0.356$, $p > 0.05$).

Body weight

Body weight, presented in Table 4.2, was significantly increased after chronic treatment with citalopram ($F_{2,28} = 3.543$, $p < 0.042$). Post hoc testing revealed that the relative body weight of animals treated with 20 mg/kg citalopram was significantly higher than that of control animals.

16 hole board

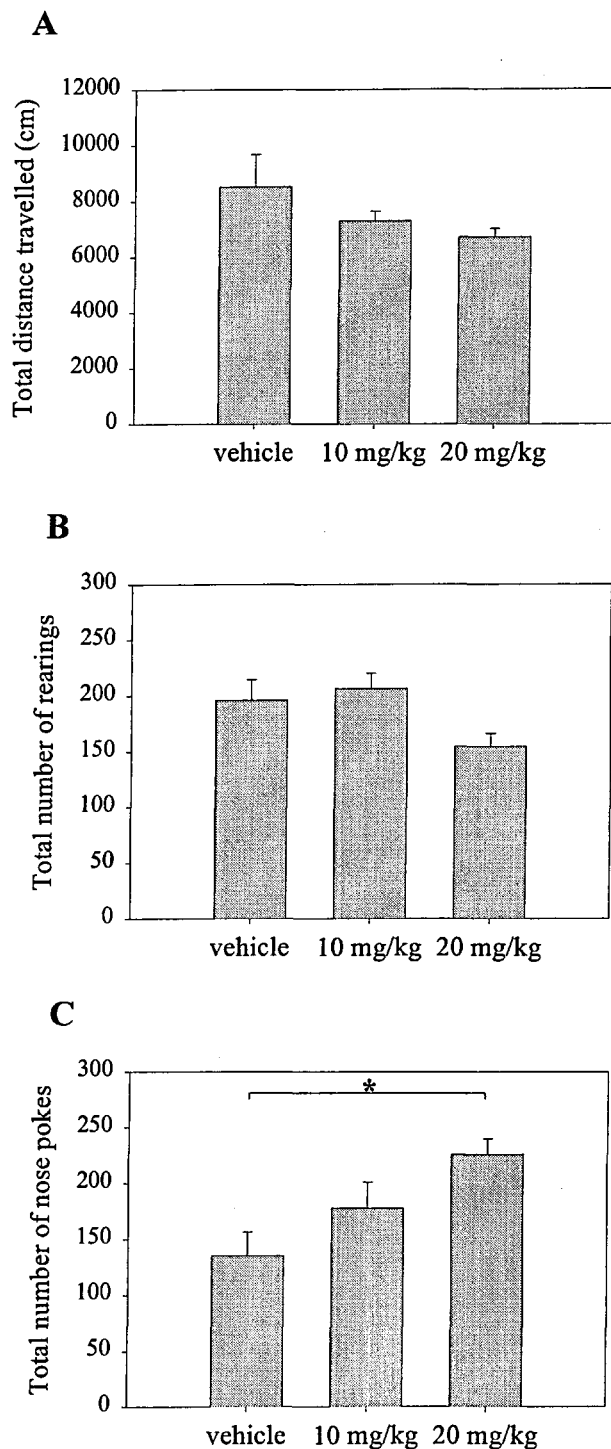


Figure 4.2 Effects of chronic citalopram treatment on hole board exploration: total distance travelled (A), total number of rearings (B) and total number of nose pokes (C) are shown. Data are presented as means, with error bars denoting SEMs; *, $p < 0.05$ significant differences between groups.

Conditioned Fear

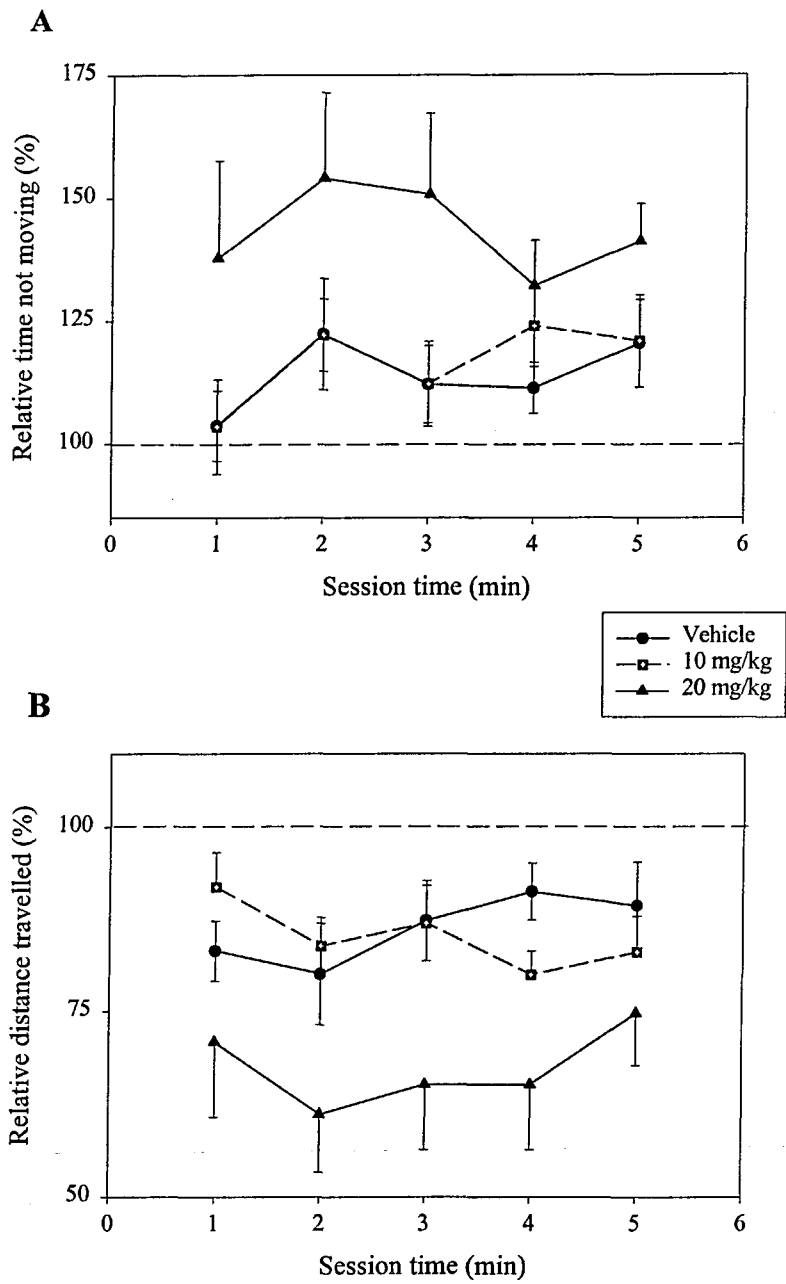


Figure 4.3 Effects of chronic citalopram treatment on conditioned fear: relative time not moving (A) and relative distance travelled (B) are shown. Data are presented as means, with error bars denoting SEMs.

Table 4.2 Effect of chronic citalopram treatment on body weight.

Treatment	Relative body weight
Vehicle	105 ± 1.14
10 mg/kg	109 ± 1.63
20 mg/kg	110 ± 1.20 *

*, $p < 0.05$ significantly different compared to controls

Discussion

The results from this experiment show that chronic treatment with citalopram results in an increased emotional response to the cue in the conditioned fear paradigm. On the plus maze, citalopram increases the time spent on the central square and tends to decrease open arm arm entries, suggesting increased anxiety-related behaviour. In addition, a similar trend in increased anxiety-related behaviour was found in the light dark box, however, failed significance. No effects of treatment on anxiety-related behaviour were found in the open field, novel object exploration paradigm and Vogel conflict task. Furthermore, citalopram treatment resulted in an increase in exploratory behaviour in terms of nose pokes made on the hole board, whereas a mild reduction in locomotion was found. Finally, increment in body weight was higher in citalopram treated animals compared to untreated mice.

In a recent review, Griebel (Griebel, 1995) summarised 72 studies in which the effects of SSRI's on anxiety-related behaviour were studied. Of these studies, 26 reported anxiogenic effects, 27 were reported to be ineffective, and 19 reported

anxiolytic effects. Studies were compared in which rats and mice were used, drugs were administered acutely or chronically, and a variety of tests were used. The data presented here suggest that the effects on anxiety-related behaviour of chronic administration in mice are task dependent, e.g. results in an anxiogenic profile in some tasks, whereas it is without effect in others.

This is in line with the hypothesis that different types of anxiety-related behaviour can be distinguished in rodents, and underlines that the use of a battery of anxiety tasks is advantageous to study aspects of anxiety.

Long-term treatment with citalopram induced a two-sided profile on anxiety-related behaviour: No effect on anxiety-related behaviour was found in light dark exploration task, novel object exploration and Vogel conflict task. In contrast, the increased time spent on the central platform and the overall effect of citalopram on open arm entries on the elevated plus maze suggest increased anxiety-related behaviour after chronic citalopram treatment. Likewise, the increased time spent on the platform of the elevated plus maze also suggests increased anxiety-related behaviour (Holmes, Rodgers, 1999). One possible explanation for this discrepancy found in the different tasks could be the difference in time of testing in connection with the treatment schedule. Given that long-term treatment would have failed to result in steady state drug levels, drug levels might have been higher during plus maze testing at night than during testing in the other tasks during the day. However, it should be noted that a significant effect of treatment was found in the conditioned fear paradigm, also suggesting increased anxiety-related behaviour. Furthermore, a similar tendency in treatment induced reduction of locomotor activity was found in

all paradigms, which reached significance in the light dark exploration paradigm, again suggesting treatment effects being independent of time of testing. Thus, the effects of citalopram on anxiety-related behaviour seems to depend on task and not on time of testing.

Since responses in the Vogel conflict and conditioned fear paradigm are potentially confounded by alterations in pain threshold, nociception was tested on the hot plate, but results suggest that performance in the Vogel conflict and conditioned fear paradigm was not affected by altered pain threshold. Possible alteration in learning or memory abilities, however, cannot be ruled out as potential interfering factors in the conditioned fear paradigm.

The present data are in contrast to clinical findings in patients suffering from anxiety disorders. One could speculate that SSRI's have anxiolytic properties only in animals with increased emotionality. Several preclinical studies support this hypothesis. For example, Keeney and Hogg (1999) recently reported that chronic treatment with citalopram is ineffective in non-stressed mice, whereas it has anxiolytic-like properties in chronically stressed animals. Furthermore, pre-treatment with the SSRI fluoxetine diminished social defeat-induced hypophagia, weight loss and anxiety without affecting these variables in control animals (Berton et al., 1999). Along similar lines, it has been demonstrated that chronic stress can evoke an immunosuppressive response, and fluoxetine has been reported to attenuate the adverse effects of stress, without significantly affecting the immune system in unstressed mice (Freire-Garabal et al., 1997). In the present study, we have studied the effects of chronic treatment with citalopram in non-stressed animals. In order to

model anxiety-related disorders with more resemblance to what is seen in the clinic, it might be of advantage to use subjects with increased emotionality. Possible methods could be chronic stress models, or the use of mouse mutants which display enhanced anxiety disorder, e.g. CRH overexpressing mice (Stenzel-Poore et al., 1994) (Heinrichs et al., 1997b).

Locomotor activity was not significantly affected by treatment in the majority of tasks, but a comparable tendency for hypolocomotion was found in all tests following treatment with citalopram. Moreover, in the light dark exploration paradigm, locomotor activity was significantly decreased. These results suggest that long-term treatment with citalopram induce mild hypoactivity. Conversely, citalopram dose-dependently increased the number of nose pokes made in the hole board paradigm. Nose poking has been suggested to be a kind of exploratory behaviour which is independently of locomotor activity in rats (Escorihuela et al., 1999) and mice (Rogers et al., 1999). This in turn indicates increased exploratory activity following chronic treatment with citalopram.

Furthermore, the effects of citalopram on body weight merit discussion. In the present study, we observed a dose-dependent increase in body weight. We have found similar effects on body weight after chronic treatment with the SSRI paroxetine in another mouse strain (Van Gaalen and Steckler, unpublished result). This is in contrast with another preclinical study, in which weight decreasing properties of the SSRI fluoxetine in mice and rats were shown (Yen, Fuller, 1992). In the clinic, effects of SSRIs on body weight seem to differ depending on the patient population: Chronic treatment with citalopram or sertraline did not alter body weight

in patients suffering from major depression (Stahl, 2001). On the other hand, SSRI's have been demonstrated to be beneficial in female obese patients (Pijl et al., 1991), and effective in patients suffering from bulimia nervosa and anorexia nervosa (Kaye et al., 1998; Ferguson et al., 1999). To find out whether the effects of chronic treatment with SSRI's are also dependent on other factors, as for example the emotional state of the animal, further investigation is needed.

Chapter 5

Effects of life-long overproduction of CRH on anxiety-related behaviour in mice

Introduction

Citalopram turned out to have anxiogenic properties in some murine anxiety paradigms after long-term treatment. This contrasts clinical findings, where it has been shown to have anxiolytic properties in addition to its antidepressive actions. As explanation for this discrepancy, it was suggested that citalopram might show an anxiolytic action in murine models which bear some similarities to symptoms seen in patients suffering from anxiety disorders (see Chapter 4). Because dysregulation in CRH systems has been suggested to play a role in a variety of stress related psychiatric disorders, such as anxiety and depression, a mouse mutant with a disrupted CRH system might be of interest. Therefore, a mouse overproducing CRH was chosen.

This mouse has been developed in order to examine the effects of chronic CRH excess (Stenzel-Poore et al., 1992). These animals have been reported to display

enhanced anxiety-related behaviour when tested on the elevated plus maze (Stenzel-Poore et al., 1994) or in a light dark exploration paradigm (Heinrichs et al., 1997b), both paradigms measuring innate anxiety. It is unclear whether overproduction of CRH also results in alteration in other types of anxiety tasks. Therefore, the present set of experiments extended these studies and included tests of innate anxiety (light dark exploration), but also conflict behaviour (Vogel conflict paradigm), a learned response (conditioned fear), exploration of a novel open field, hole board exploration, and forced swimming

In fact, hypoactivity of transgenic mice exploring a novel environment has been reported (Stenzel-Poore et al., 1994). However, studies investigating effects of central administered CRH revealed that the effect on locomotor activity depend on novelty/familiarity of the environment (for review see: Dunn and Berridge, 1990; Steckler, Holsboer, 1999). Therefore, we also investigated the effects of CRH overproduction in the home cage, in a novel and a familiar open field. Furthermore, sensorimotor performance was tested on a rotarod.

Several studies have reported the abilities of benzodiazepines to attenuate the anxiogenic effects of central CRH administration (for review see Dunn and Berridge, 1990). Conversely, local CRH administration increased GABA release via receptors present on GABA-containing neurons (Sirinathsinghi and Heavens, 1989), suggesting possible interactions between the GABA / benzodiazepine and CRH systems. Furthermore, it has been shown that chlordiazepoxide, a benzodiazepine with both anxiolytic and amnestic properties, attenuated a water maze place learning deficit in transgenic mice overexpressing CRH (Heinrichs et al., 1996). The authors

argued that the beneficial effects of the drug could best be explained by its effects on heightened anxiety/arousal, which could have confounded water maze performance in transgenic mice. To further dissociate the effects of CRH overproduction on open field exploration, the effect of diazepam on locomotor activity was studied.

Material and Methods

Animals

CRH transgenic mice, originally generated on C57BL/6 × SJL background in one line of mice by M. P. Stenzel-Poore (1992), were backcrossed onto C57BL/6 for seven generations. 13 Heterozygous male transgenic mice and 17 male wildtype littermates were used in experiment 1, 12 heterozygous male transgenic mice and 12 male wildtype littermates were used in experiment 2, all aged between 2 to 3 months, and bred and reared in the animal house of the Max Planck Institute. The CRH transgene was composed of the rat genomic CRH gene, with the 5' regulatory region being replaced by the murine metallothionein-1 gene. The 3' untranslated region of the human growth hormone gene was ligated to the 3' end of the CRH gene in order to ensure adequate RNA processing of the fusion gene (Stenzel-Poore *et al.*, 1992). Animals were housed individually and maintained on a 12:12 h light/dark cycle (lights on at 06:00 hours) with *ad lib* water and food unless mentioned differently in experiment description.

Experiment 1

After placing the animal in a confinement for 1 min, spontaneous behaviour was analysed in the home cage for ten minutes. The total distance travelled over the full

session was analysed with a video tracking system (VideoMot2, TSE, Germany). Furthermore, time spent rearing, grooming and digging in the bedding were scored manually over two 5 min bins. Next, animals were tested in the light dark exploration paradigm. Locomotor activity was assessed in a 30 minutes open field session without prior habituation (novel open field). The first 5 min of the session were analysed on time spent and relative distance travelled in the centre of the open field as a measure for anxiety-related behaviour.

Conflict behaviour was investigated in the Vogel conflict task. Further, exploratory behaviour was analysed in the hole board paradigm, after which conditioned fear was studied. Furthermore, pain threshold was assessed in using the hot plate test, and 24-hour water intake was measured. Sensorimotor skills were assessed by testing the animals on a rotarod for three sessions, each spaced with 2 hours. Finally, animals were exposed to inescapable swim stress. Immobility time during the last three min of testing was scored. At least 5 days lapsed between tests.

Experiment 2

New groups of animals were repeatedly tested in an open field (same apparatus used for open field testing in experiment 1). Animals were tested once a day. First, animals were habituated for 5 sessions, one 30 min session per day (familiar open field). Then, animals were re-tested in the open field after receiving saline or diazepam (0.5, 2.5 and 7.5 mg/kg, intraperitoneal (i.p.)). Each dose of drug was compared with saline vehicle utilising a Latin-square design (the sequence of treatments were randomised). Between treatment sessions, animals were tested for one session without vehicle/drug administration. Relative distance travelled after

treatment was calculated, with the distance travelled at habituation session 5 taken as baseline.

To control for possible alterations in water intake in a novel environment, animals (experiment 2) were tested for two sessions spaced by one week. Prior to testing, animals were water deprived for 24 hours. The testing procedure on session 1 was similar as in experiment 1, except that no shock was delivered. Session 2 was similar to the original lick suppression protocol. The percentage of response inhibition was calculated from the number of episodes completed during sessions 1 and 2 in order to control for motivational differences. Furthermore, the relative change in latency to start the session was calculated from the latencies obtained during sessions 1 and 2.

Conditioned fear

The same apparatus and procedure was used as in experiment one, except now animals were exposed to the test box, 1 hour after conditioning.

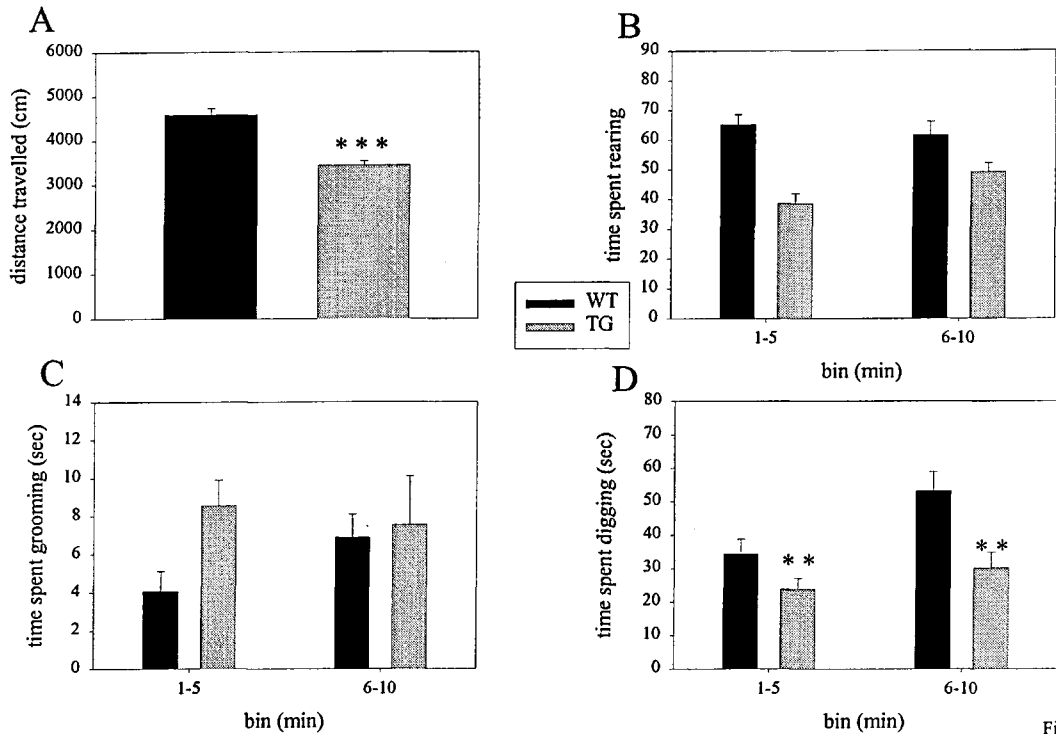
At least one week lapsed between tests.

Results

Examination of spontaneous behaviour

Locomotor activity in the home cage, as measured by total distance travelled, was significantly decreased in transgenic animals ($F_{1,28} = 35.62$, $p < 0.001$; Figure 5.1A).

spontaneous behaviour



Figure

Figure 5.1 Effects of overproduction of CRH on spontaneous behaviour in the home cage. Distance travelled (A) was analysed over 10 min. The times spent rearing (B), grooming (C) and digging (D) were analysed over two successive 5-min time bins. Data are presented as means, with error bars denoting SEMs; group differences: ** $p < 0.01$; *** $p < 0.001$.

Overall, time spent rearing was also decreased in transgenics ($F_{1,28} = 35.62$, $p < 0.001$; Figure 5.1B). Although a genotype x bin interaction was found for the latter measure ($F_{1,28} = 6.77$, $p = 0.015$), post hoc testing failed to reveal significant differences. No genotype x bin interaction was found in time spent grooming ($F_{1,28} = 1.28$, $p > 0.05$; Figure 5.1C), but the overall time spent grooming tended to be increased in transgenic animals ($F_{1,28} = 3.23$, $p = 0.083$). Furthermore, transgenic mice spent less time digging than wildtype animals ($F_{1,28} = 8.03$, $p = 0.008$; Figure 5.1D), but no genotype x bin interaction was found ($F_{1,28} = 2.94$, $p > 0.05$).

Light dark exploration

Transgenic animals showed reduced activity compared to wildtype animals in term of distance travelled ($F_{1,28} = 23.55$, $p < 0.001$; Figure 5.2A) and number of rearings made ($F_{1,28} = 12.93$, $p = 0.010$; Figure 5.2B). Furthermore, latency to enter the light compartment was increased in transgenic mice ($F_{1,28} = 12.65$, $p = 0.001$; Figure 5.2C) which also spent less time in lit area ($F_{1,28} = 11.31$, $p = 0.002$; Figure 5.2D), indicating increased anxiety-related behaviour. Moreover, moving time and relative distance travelled in the light compartment were decreased in transgenic animals ($F_{1,28} = 15.91$, $p < 0.001$ and $F_{1,28} = 13.02$, $p = 0.001$; Figures 5.2E, F), suggesting that the reduced exploration of the illuminated compartment was not due to altered locomotor activity.

Novel open field

Overall, mutants travelled a significantly shorter distance ($F_{1,28} = 13.94$, $p < 0.001$; Figure 5.3A), but no genotype x bin effect was found ($F_{5,140} = 2.32$, $p > 0.05$), although visual inspection of the data suggested that the major differences were seen during the first 10 min of exploration. Parameters possibly indicating anxiety-related behaviour were analysed during the first five minutes, but no significant differences were found in terms of time spent ($F_{1,28} = 2.18$, $p > 0.05$; Figure 5.3B) and relative distance travelled ($F_{1,28} = 2.07$, $p > 0.05$; Figure 5.3C) in the centre of the open field.

Familiar open field: effects of diazepam

There was a significant effect of session during the five habituation session in distance travelled ($F_{4,88} = 14.68$, $p < 0.001$): activity of animals was higher during session 1, but did not differ thereafter. No effect of genotype ($F_{1,22} = 0.12$, $p > 0.05$),

Light Dark Exploration

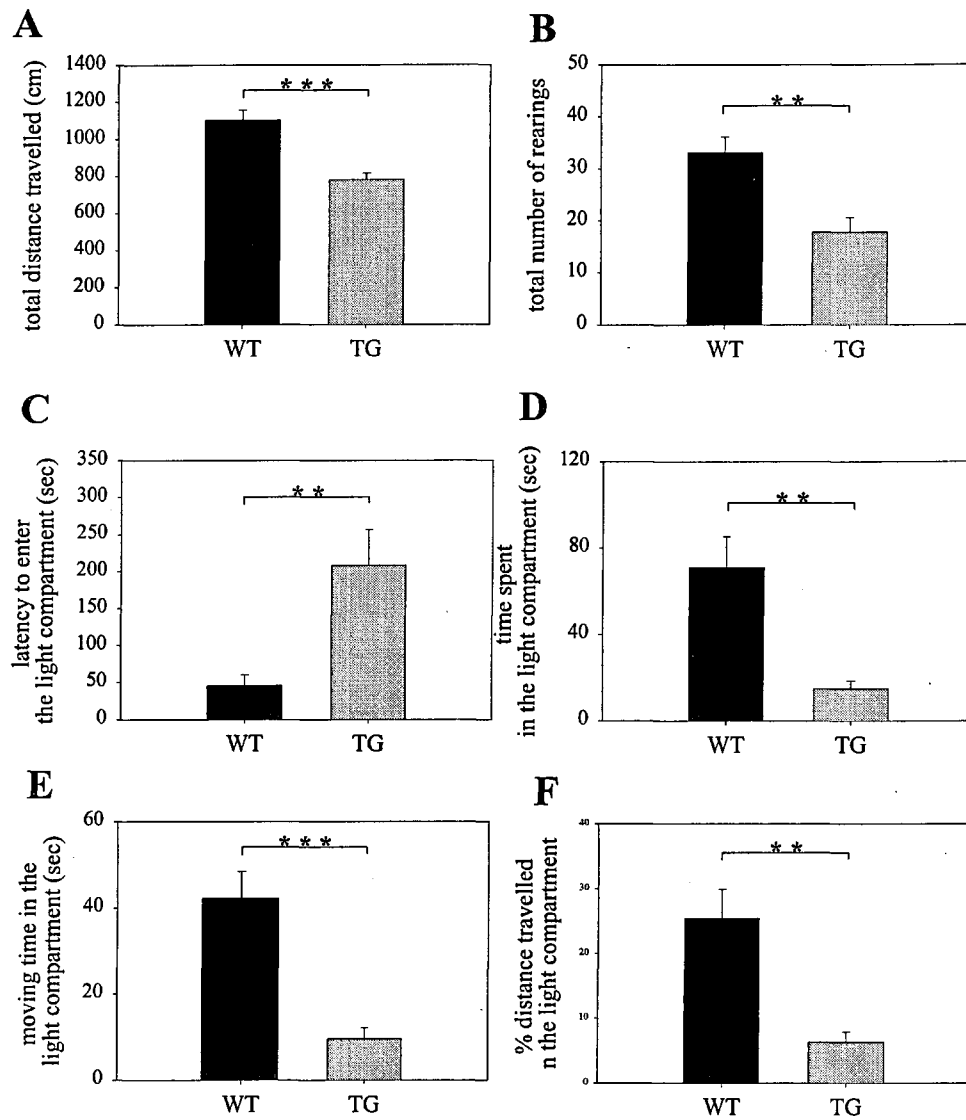


Figure 5.2 Effects of overproduction of CRH on light dark exploration: the total distance travelled over 5 min (A), the total number of rearings (B), latency to enter the light compartment (C), the time the animal spent in the illuminated part (D), the time the animal spent moving in the illuminated part (E), the relative distance travelled in the light (E) and the relative distance travelled in the illuminated area (F) are shown. Data are presented as means, with error bars denoting SEMs; **, $p < 0.01$; ***, $p < 0.001$.

Novel Open Field

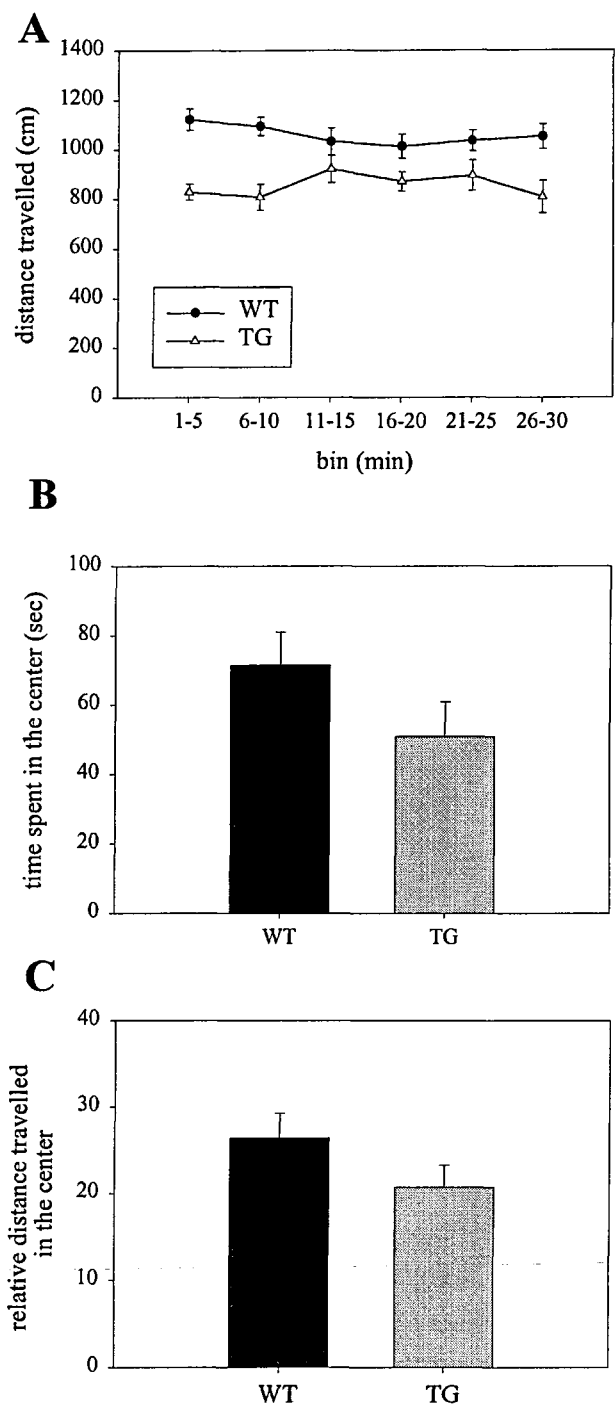


Figure 5.3 Effects of overproduction of CRH on behaviour in a novel open field: the total distance travelled over 30 min (A), the time spent in the centre of the open field during the first 5 min (B) and the relative distance travelled in the centre during the first 5 min (C) are shown. Data are presented as means, with error bars denoting SEMs.

or genotype x session interaction ($F_{4,88} = 0.95$, $p > 0.05$) was found during this habituation stage (data not shown). Diazepam administration dose-dependently reduced the relative distance travelled in both groups ($F_{3,84} = 13.47$, $p < 0.001$; Figure 5.4). Furthermore, a group difference was found after treatment ($F_{1,84} = 25.15$, $p < 0.001$), although no genotype x treatment effect was found ($F_{3,84} = 2.03$, $p > 0.05$).

Hole board exploration

Again, transgenic mice showed an overall decreased locomotor activity, comparable to what has been observed in the open field ($F_{1,28} = 14.05$, $p < 0.001$; data not shown). However, genotypes failed to differ in the number of nose pokes made ($F_{1,28} = 1.47$, $p > 0.05$; Figure 5.5). No genotype x bin interaction was seen for this measure ($F_{5,140} = 2.07$, $p > 0.05$). However, visual inspection of the data suggested a possible genotype effect on the number of nose pokes made in the early testing phase. Therefore, the first 5 min of exploration were analysed separately, which indicated that transgenic mice made significantly less nose pokes during this period compared to wildtype mice ($F_{1,28} = 4.77$, $p = 0.037$), whereas no significant group differences were found in the number of rearings.

Familiar Open Field

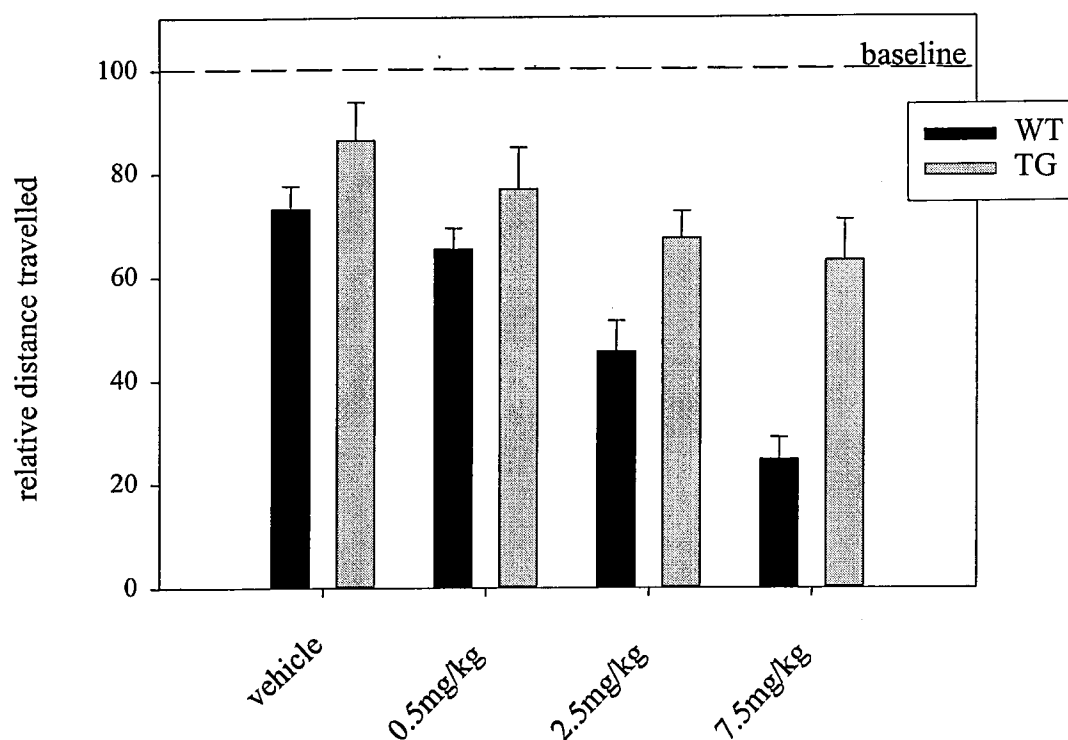


Figure 5.4 Effects of overproduction of CRH in a familiar open field: The relative distance travelled from baseline (dotted line) after vehicle / diazepam treatment is shown. Data are presented as means, with error bars denoting SEMs

Lick suppression

Transgenic mice showed a decreased latency to start the session compared to controls ($F_{1,27} = 6.72$, $p = 0.015$; Figure 5.6A). The number of finished episodes, however, was not significantly different between groups ($F_{1,27} = 0.17$, $p > 0.05$; Figure 5.6B).

Repeated exposure to the lick suppression chamber in experiment 2 revealed that the number of completed episodes decreased from session 1 (without shock) to session 2 (with shock; wildtype $25.8\% \pm 9.9$, transgenic $21.2\% \pm 11.9$). However, no effects of genotype were seen when the relative number of episodes were considered ($F_{1,17} = 98.78$, $p > 0.05$). The latency to start a session was decreased upon re-testing (wildtype $17.2\% \pm 21.7$, transgenic $43.6\% \pm 13.5$), but again, no significant effect of genotype was seen ($F_{1,17} = 1.01$, $p < 0.050$), suggesting that lack of difference in conflict behaviour between mutants and wildtype animals is not due to altered motivational drive.

Conditioned fear

The relative time not moving was analysed after re-exposure of the cue. A significant effect of bin was found when animals were re-tested 1 h after conditioning (experiment 2; $F_{4,72} = 13.94$, $p < 0.001$; Figure 5.7). Overall, the relative resting time after re-exposure to the cue was lower in transgenic animals compared to wildtype mice ($F_{1,18} = 5.91$, $p = 0.026$), but no genotype x bin interaction was observed ($F_{4,72} = 46.98$, $p > 0.05$).

Re-exposure to the cue 24 h after conditioning also revealed an effect of time bin (experiment 1; $F_{4,100} = 37.79$, $p < 0.001$; Figure 5.8). No effect of genotype ($F_{1,25} = 0.49$, $p > 0.05$) or a genotype x bin interaction ($F_{4,100} = 1.86$, $p > 0.05$) were seen.

16 Hole Board

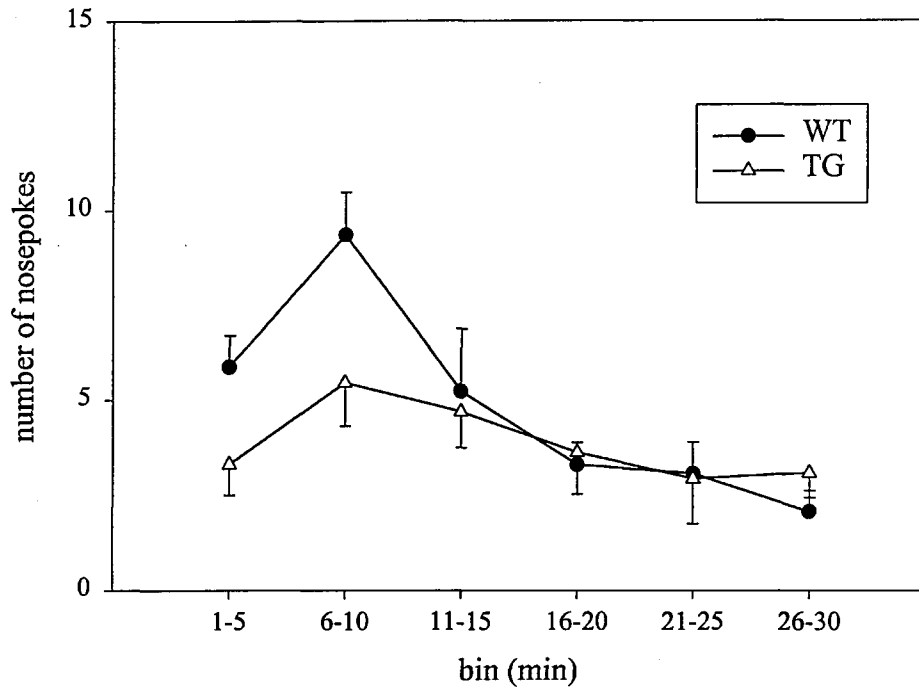


Figure 5.5 Effects of overproduction of CRH on hole board exploration: number of nose pokes made during a 30 min session are shown. Data are presented as means, with error bars denoting SEMs.

Hot plate

During the hot plate test, no jumping was observed. Latencies to feet licking were not significantly different between groups ($F_{1,27} = 2.32$, $p > 0.05$; Table 5.1).

Vogel Conflict Task

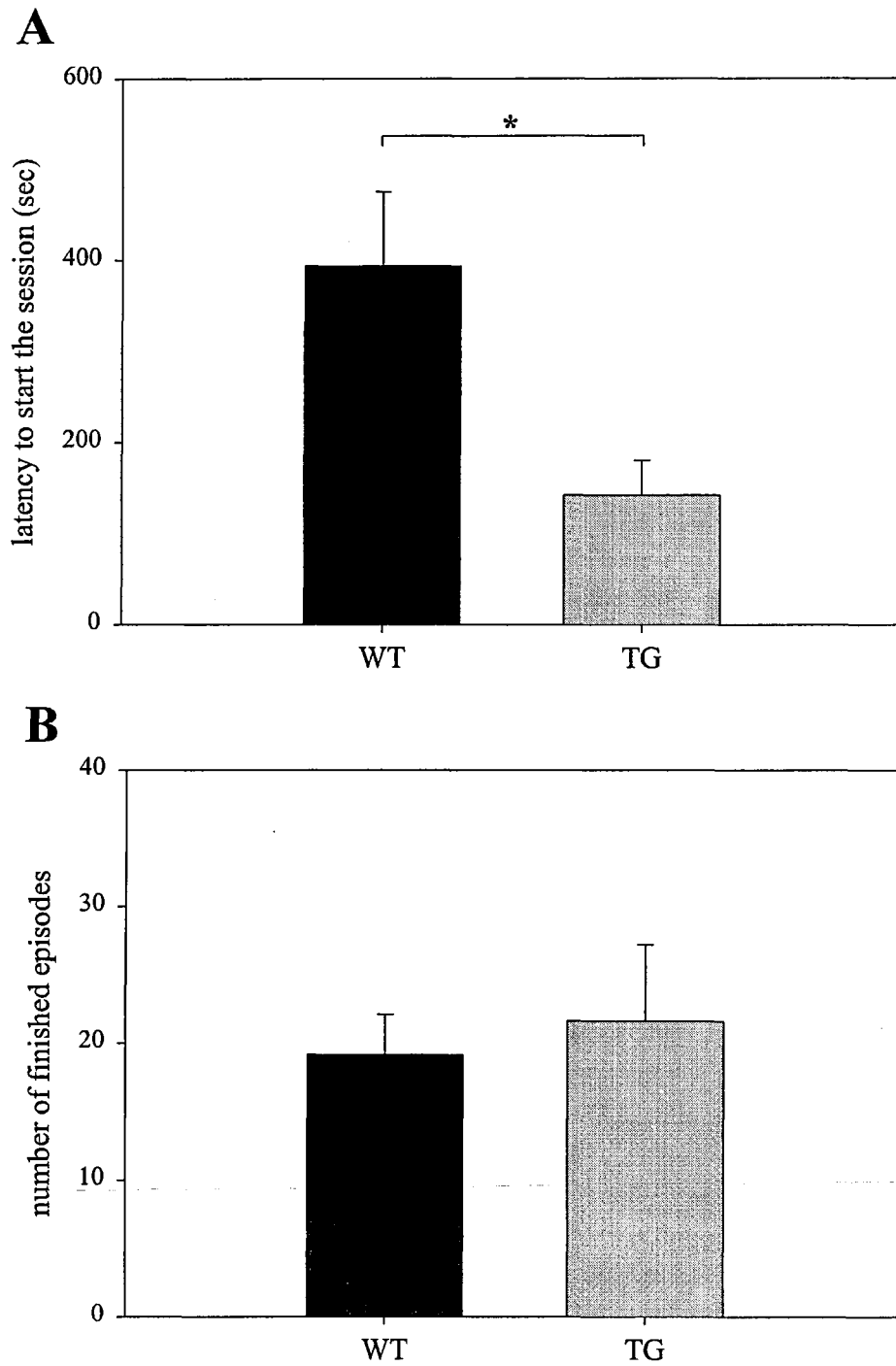


Figure 5.6 Effects of overproduction of CRH on conflict behaviour: latency to start the session (A) and number of finished episodes (B), are shown. Data are presented as means, with error bars denoting SEMs; *, $p < 0.05$.

Conditioned Fear

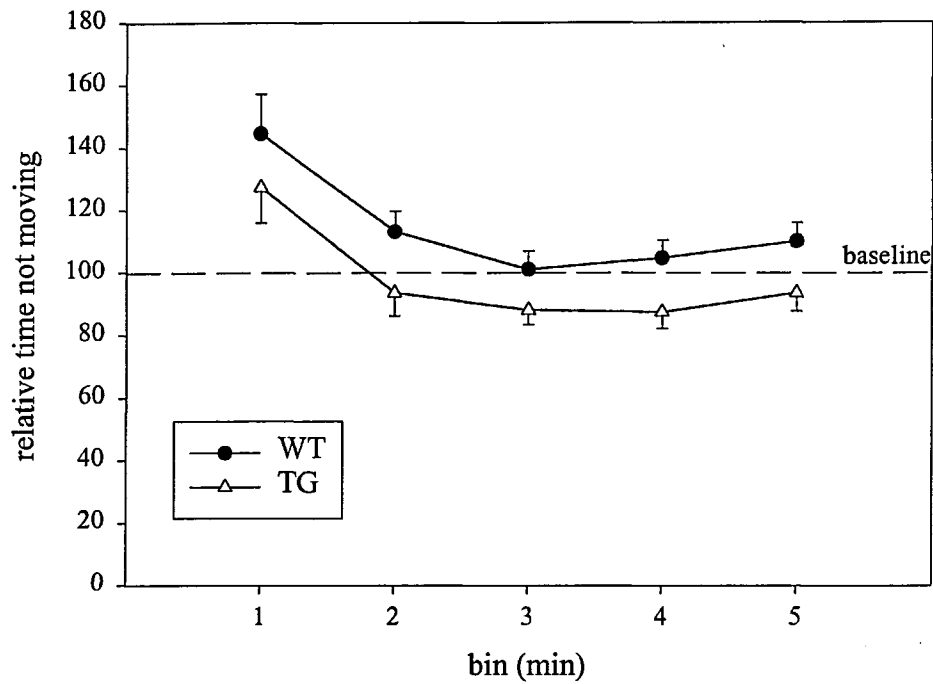


Figure 5.7 Effects of overproduction of CRH on conditioned fear 1 h after conditioning : change in the time not moving during re-exposure to the light relative to baseline (dotted line) is shown. Data are presented as means, with error bars denoting SEMs.

Water intake

Twenty-four hour water intake was significantly higher in transgenic animals compared to wildtype ($F_{1,28} = 49.72$, $p < 0.001$; Table 5.1).

Conditioned Fear

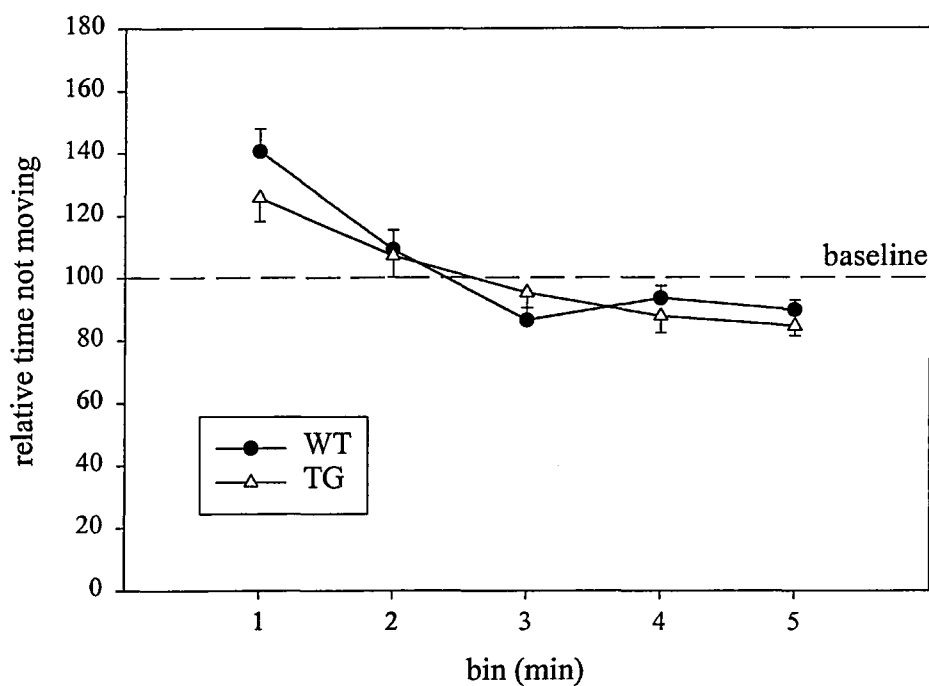


Figure 5.8 Effects of overproduction of CRH on Conditioned fear 24 h after conditioning: change in the time not moving during re-exposure to the light relative to baseline (dotted line) is shown. Data are presented as means, with error bars denoting SEMs.

Table 5.1: Effects of overproduction of CRH on pain threshold and water intake.

	WT	TG
Pain threshold	11.9 ± 2.4	13.6 ± 3.5
Water intake	3.8 ± 0.1	5.8 ± 0.3***

Data are presented as means ± SEMs; ***, $p < 0.01$, significant different to the other strains.

Rotarod

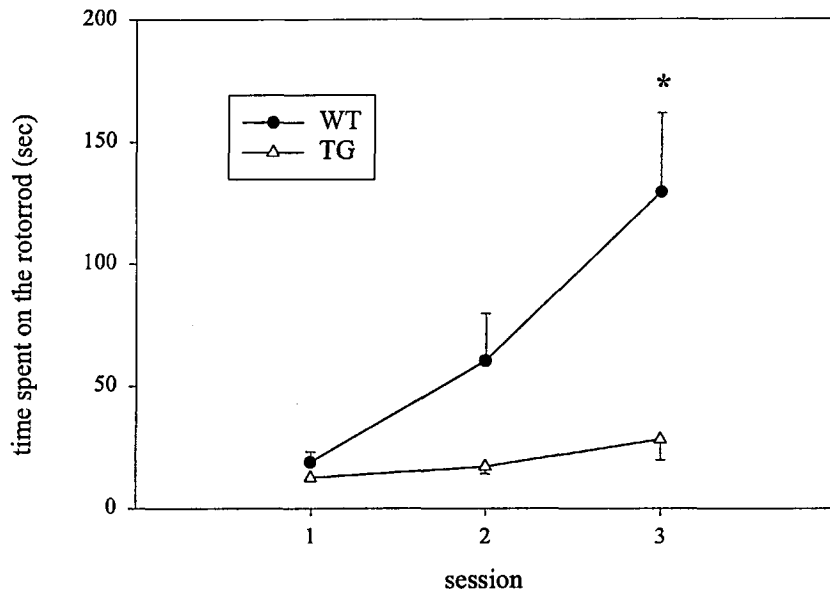


Figure 5.9 Effects of overproduction of CRH on rotarod performance: time spent on the rotarod in successive sessions is shown. Data are presented as means, with error bars denoting SEMs; *, $p < 0.05$.

Sensorimotor skills

There was an effect of bin ($F_{2,52} = 7.62$, $p = 0.004$; Figure 5.9) on time spent on the rotarod, indicating that mice improved performance over sessions. There was also a genotype x session effect ($F_{2,52} = 4.23$, $p = 0.034$), and an overall genotype effect was observed ($F_{1,26} = 6.17$, $p = 0.020$), with transgenic mice being impaired relative to wildtype animals. Further post hoc testing indicated that the groups only differed in the third session ($P < 0.05$).

Inescapable Swim Stress

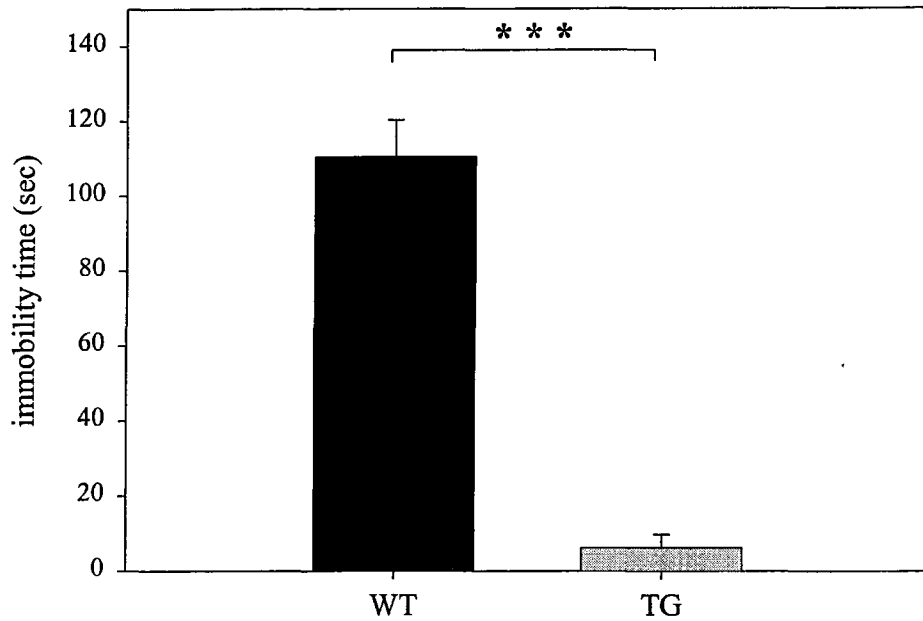


Figure 5.10 Effects of overproduction of CRH on inescapable swim stress: immobility time during the last three minutes of testing is shown. Data are presented as means, with error bars denoting SEMs; ***, $p < 0.001$.

Inescapable swim stress

Transgenic animals spent less time immobile compared to controls ($F_{1,26} = 67.58$, $p < 0.001$; Figure 5.10).

Discussion

The main findings of the present study are that transgenic mice displayed increased anxiety-related behaviour in some, but not all, anxiety tasks. Furthermore, these animals were hypoactive in novel environment, whereas no difference in locomotor activity was found after being habituated to a test environment. Finally, CRH overexpressors were less susceptible to the sedative effect of diazepam.

Transgenic mice showed increased anxiety-related behaviour in the light dark exploration task, which is in line with finding from Heinrichs and colleagues (1997b). The anxiety-like effects observed were not likely to be due to hypoactivity, as relative activity in the illuminated compartment in the light dark exploration task was also reduced. A tendency of increased anxiety-related behaviour in transgenic mice was found in terms of time spent grooming in the spontaneous behaviour paradigm and in anxiety parameters assessed in the open field. Results from the Vogel conflict task did not reveal a difference between CRH transgenic mice and wildtype littermates, but this lack of difference could be due to altered motivation as transgenic mice have an increased water intake. The results obtained in the second experiment, in which relative shock-induced response suppression was assessed, also failed to reveal a difference between groups, which would argue against this possibility. The unaltered conflict behaviour displayed by CRH overexpressing mice is in contrast to the reported anxiogenic effects of acute centrally administered CRH in conflict tasks (Britton et al., 1985, 2000). This suggests that challenging of the CRH system can affect conflict behaviour, whereas chronic hyperactivation does not

alter conflict behaviour, in contrast to anxiety-related behaviour in innate test of anxiety as for example the light dark exploration task.

In addition, no genotype differences were found in conditioned fear when tested 24 h after conditioning. Strikingly, a reduced emotional response of the transgenics to the CS was found when tested 1 hour after conditioning. However, the conditioned fear experiment presented in Chapter 3 show that the emotional response, in terms of locomotor inhibition, occurs in the first min after representation of the cue. Visual inspection of the data of the fear conditioning experiments presented here, indicate that the emotional response is comparable when measured 24 hours or 1 hour after training. This suggests that conditioned fear is not different in CRH overexpressors. However, an effect of CRH overproduction on learning and memory cannot be excluded. For example, several studies have shown that centrally administered CRH or its displacement form CRH-binding protein enhances memory in multiple learning tasks (Koob, Bloom, 1985; Liang, Lee, 1988; Behan et al., 1995; Heinrichs et al., 1997), whereas i.c.v. administration before the memory test seems to impair memory (Diamant, de Wied, 1993). More recently, Radulovic and colleagues (1999) showed that centrally administered CRH can either enhance or impair learning depending on the region of administration, and the authors suggested that CRH acted on memory consolidation. These findings make it difficult to speculate whether life-long overproduction of CRH would alter learning in the present experiment. This question was assessed by Heinrichs and colleagues (1996), who reported that CRH overproducing mice show impaired performance in a place navigation task. However, this deficit was attenuated by administration of the benzodiazepine chlordiazepoxide, whilst treatment impaired the performance of control animals. The

authors postulate that deficits in place navigation in CRH overproducing mice might not be due to impaired spatial learning / memory per se, but is possibly due to the increased arousal / anxiety. In addition, the results from both fear conditioning experiments indicate that mutants can learn to associate CS with the US, however, no increased emotional response, as may be expected, was found.

Footshock might, however, be experienced as more aversive by transgenics. This gains additional relevance in the Vogel conflict, which might also be influenced by nociception. However, results of the hot plate experiment suggest that nociception is not altered in transgenics. This is in line with the failure of CRH to affect nociception on the hot plate and in the tailflick test when administered centrally (Britton et al., 1985; Sherman, Kalin, 1986).

Interestingly, the number of nose pokes made in the hole board was only reduced during the early testing phase. Nose pokes are believed to be an index for exploratory behaviour, and can be assessed independently of locomotor activity in rats (Escorihuela et al., 1999) and mice (Rogers et al., 1999). It is possible that the reduction in number nose pokes made were confounded by altered anxiety. For example, it has been shown that the number of hole visits on a hole board can be increased after administration of anxiolytic compounds, whereas anxiogenic

compounds as well as restraint stress decrease the number of visits in mice (Takeda et al., 1998). Therefore, the reduction in exploratory behaviour (nose pokes) during the early testing phase might be due to increased anxiety displayed by transgenic mice.

Mice overexpressing CRH were more active compared to wildtype littermates when exposed to inescapable swim stress. Similar testing conditions have been used by Heinrichs and co-workers (Heinrichs et al., 1996), but in these experiments latency to immobility was measured. No genotype-dependent difference was found, which was interpreted as no difference in latency to tire. However, acute centrally administered CRH has been reported to reduce immobility time (Butler et al., 1990). The authors proposed that these measures possibly reflect possible anxiety. This is in line with findings from Ferre and colleagues (1964), who demonstrated that murine anxiety-related behaviour on the plus maze and immobility time in a forced swimming task show a reversed correlation. In addition, data presented in Chapter 3 point in the same direction. Therefore, it might be that the decreased immobility time of the transgenic mice might be due to increased anxiety.

Difference in locomotor activity was depending on the context of the testing environment. Thus, CRH transgenic mice displayed hypoactivity activity when tested in a novel environment (open field, light dark exploration, and hole board exploration) or under conditions of enhanced arousal in the modified home cage. This is in agreement with previous work from Stenzel-Poore and colleagues (1994), showing that CRH overexpressors are hypoactive in a novel environment. Similar results have been found after acute i.c.v. administration of CRH (Sutton et al., 1982;

Sherman and Kalin, 1986; Britton et al., 1986; Conti et al., 1994; Jones et al., 1998). The hypolocomotor activity of transgenics, seen when tested in the novel environments, was no longer apparent when animals were habituated to the test conditions in the familiar open field. This contrasts the effects of acute CRH on locomotor activity, in a familiar environment, where acute CRH administration increases activity (Sutton et al., 1982; Sherman and Kalin, 1986; Britton et al., 1986).

Also of note, transgenic animals did not display hypoactivity under forced conditions. For example, on the rotarod, both groups performed equally well during the first session. However, wildtype animals improved performance over sessions, in contrast to transgenic mice. One possibility is that CRH disrupts performance at the level of the cerebellum, an area which plays an important role in fine locomotor function and in which strong expression of CRHR1 receptors has been reported (Chalmers et al., 1995; Bishop et al., 2000).

Taken together, overexpression of CRH induces effects that are in part comparable to the effects of acute CRH administration, whereas others are not. Such differences between acute and long-term CRH exposure may be due to CRH-induced alteration in other neurotransmitter system.

Administration of diazepam in the familiar open field dose-dependently reduced locomotor activity. However, transgenics were less susceptible to the sedative effects of diazepam. Diazepam enhances GABA neurotransmission by acting on the GABA_A receptor. Other agents that have such property are barbiturates, benzodiazepines, steroid anaesthetics and ethanol (see for example Zorumski and

Isenberg, 1991). Barbiturates and benzodiazepines are well known for their anxiolytic properties. Several studies have reported the abilities of benzodiazepines to attenuate the anxiogenic effects after central CRH administration (Dunn and Berridge, 1990). Furthermore, repeated benzodiazepine administration results in reduction of CRH receptor binding (Grigoriadis et al., 1989), and prenatal exposure to diazepam leads to a decrease in CRH-immunoreactivity in cells within the PVN of adult rats (Inglefield et al., 1993). In addition, local CRH administration increases GABA release via receptors present on GABA-containing neurons (Sirinathsinghji and Heavens, 1989). Taken together, this indicates that the GABA / benzodiazepine and CRH systems may interact.

This indicates, that using models of chronic CRH excess may increase the understanding in the pathology of disease in which hyperactivity of the CRH system plays a role, such as depressive illness. Furthermore, one might speculate that chronic CRH excess may increase some, but not all, aspects of anxiety. Finally, the present data again underline that a range of anxiety task is necessary to study anxiety-related behaviour in detail.

Chapter 6

Transgenic Mice Overexpressing Corticotrophin-Releasing Hormone show reduced responsiveness in plasma corticosterone after a 5-HT_{1A} receptor challenge

Introduction

Data presented in the previous chapter support the hypothesis that chronic excess of CRH increases some, but not all, aspects of anxiety. This is in line with the known anxiogenic properties of CRH. However, patients suffering from depression often show increased anxiety, and alterations in the CRH system have been found in these patients. Furthermore, the serotonergic system, in particular the 5-HT_{1A} receptor, has been implicated in the modulation of anxiety and depression. Therefore, in the

present experiment, the consequences of long-term CRH overproduction on 5-HT_{1A} receptor function were studied in transgenic mice overexpressing CRH.

5-HT_{1A} receptors serve both somatodendritic autoreceptor- and postsynaptic heteroreceptor function. In mice, 8-OH-DPAT-induced hypothermia has been attributed to activation of somatodendritic 5-HT_{1A} autoreceptors (Bill et al., 1991), while there is strong evidence to suggest that increased secretion of ACTH and corticosterone following 8-OH-DPAT administration results, at least in part, from stimulation of postsynaptic 5-HT_{1A} receptors at the level of the PVN (Pan, Gilbert, 1992).

Material and Methods

Individually housed female transgenic mice overproducing CRH (Stenzel-Poore et al., 1992) were compared to age-matched wildtype littermates (n = 5 - 9 per group, 2 to 4 months of age). In the first experiment, corticosterone levels were estimated under basal conditions (trunkblood sampling between 8.00 and 9.00 hours) and 20 min after s.c. CRH injection (30 microgram / kg).

In the second experiment, rectal temperature was recorded over 4 seconds (Thermoelectra, Pijnacker, The Netherlands), after which animals received a s.c. injection of either 8-OH-DPAT (0.5 mg/kg) or saline and were returned to their homecage for 20 min, followed by another measurement of rectal temperature. Subsequently, trunkblood was collected for corticosterone plasma estimation.

Results

Experiment 1

Plasma corticosterone levels were overall increased in transgenic mice ($F_{1,24} = 48.25$, $p < 0.001$; Figure 6.1). CRH injection resulted in an increase in plasma corticosterone levels ($F_{1,24} = 50.10$, $p < 0.001$), whereas no genotype x treatment interaction was found ($F_{1,24} = 0.36$, $p > 0.05$).

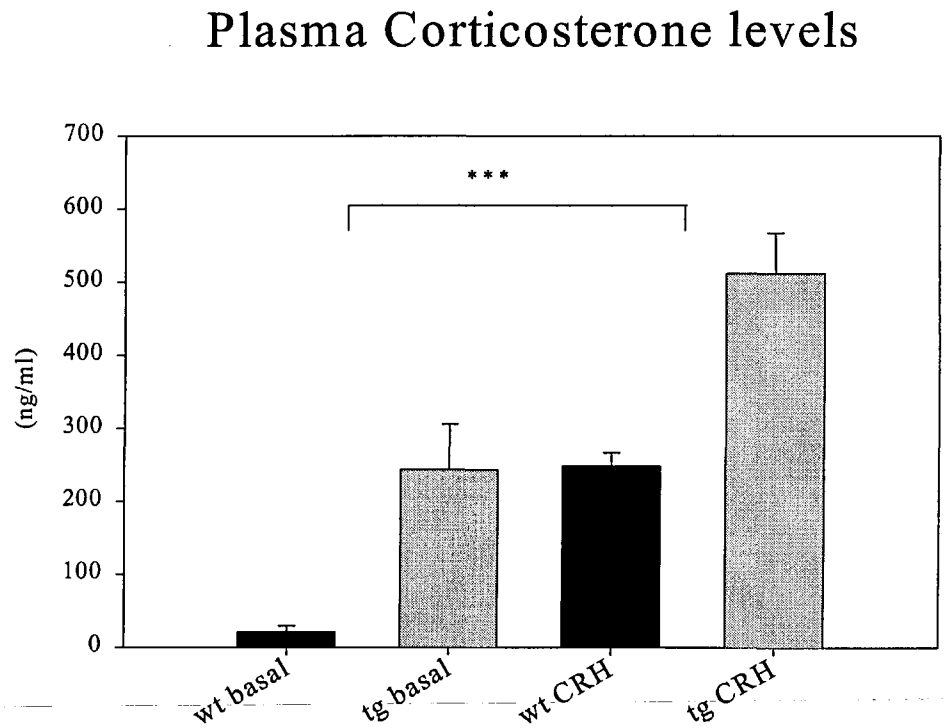


Figure 6.1 Effects of overproduction on plasma corticosterone levels, under basal conditions and 20 min after injection of CRH (30 $\mu\text{g/kg}$). wt: wildtype; tg: transgenic. Data are presented as means \pm SEMs.

Experiment 2

Body temperature was increased after injection / handling / insertion of temperature probe induced stress independently of genotype (Figure 6.2). There was a significant time x treatment effect ($F_{1,24} = 94.7$, $p < 0.001$) on body temperature after 8-OH-DPAT administration, but no significant time x genotype ($F_{1,24} = 0.12$, $p > 0.05$) or time x genotype x treatment ($F_{1,24} = 0.60$, $p > 0.05$) interactions were observed (Figure 6.2). Post hoc testing revealed that saline injection increased body temperature ($p < 0.001$), whereas 8-OH-DPAT reduced body temperature ($p < 0.001$).

Both genotype ($F_{1,24} = 22.6$, $p < 0.001$) and treatment ($F_{1,24} = 7.92$, $p < 0.010$) affected plasma corticosterone levels, ANOVA also revealed a significant genotype x treatment interaction ($F_{1,24} = 94.7$, $p < 0.010$; Figure 6.3). Additional post hoc testing indicated that plasma corticosterone levels were higher in wildtype animals treated with 8-OH-DPAT than in all other groups, while 8-OH-DPAT had no effect in CRH overexpressing mice.

Body Temperature

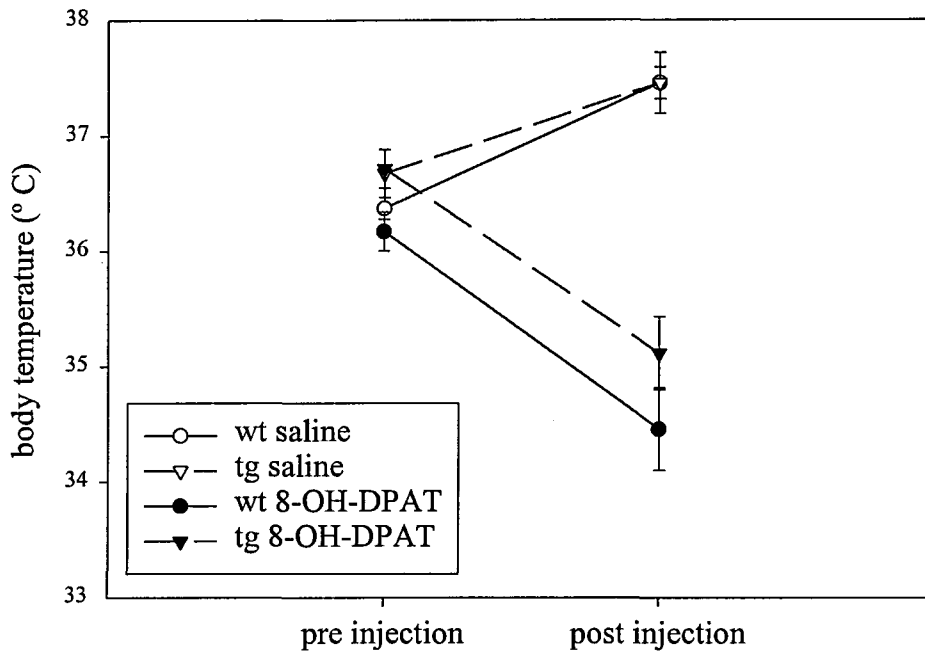


Figure 6.2 Effects overproduction of CRH on Body temperature measured under basal conditions (pre-treatment) and 20 min after treatment (post-treatment). wt: wildtype; tg: transgenic. Data are presented as means \pm SEMs.

Discussion

Central CRH administration is known to induce hyperthermic responses, and both stress and CRH-induced hyperthermia can be blocked by α -helical CRH(9-41), a non-selective CRH antagonist (Morimoto et al., 1993), suggesting a key role for CRH in stress induced hyperthermia. The fact that stress-induced hyperthermia did not differ between transgenic and wildtype mice suggests that CRH overproduction did not affect this measure, or that compensatory mechanisms are operating to maintain hyperthermic responses induced by stress in the face of CRH

Plasma Corticosterone levels

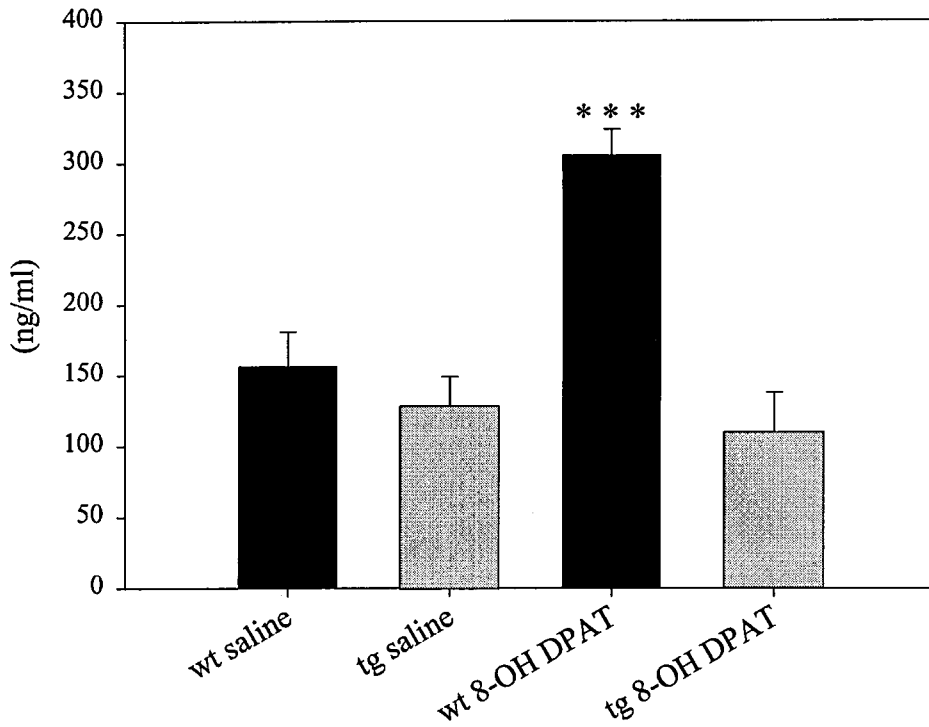


Figure 6.3 Effects of overproduction of CRH on plasma corticosterone levels, 20 min after injection of saline or 8-OH-DPAT (0.5 mg/kg). wt: wildtype; tg: transgenic. Data are presented as means \pm SEMs. *** $p < 0.001$ compared to all other three groups.

hypersecretion. 8-OH DPAT-induced hypothermia also failed to differ between groups, suggesting that presynaptic 5-HT_{1A} receptor function remained intact in transgenic mice.

Basal corticosterone plasma levels were elevated in transgenic animals, which is in line with a previous report from Stenzel-Poore and colleagues (1992). However, no differences between groups were seen on this measure following mild stress (saline administration and body temperature measurements) in the present study. This is in agreement with a recent study, reporting a blunted response of HPA axis activity

after swim stress in transgenic mice overexpressing CRH (Heinrichs et al., 1997), despite the fact that anxiety-like behaviour is increased in these animals (Heinrichs et al., 1997; Stenzel-Poore et al., 1994; Chapter 5). Thus, although the hyperthermic response remained unaffected in transgenic mice, HPA axis reactivity after mild stress was blunted in these animals, suggesting distinct mechanisms involved in adjusting to increased CRH activity.

CRH injection, at a dose that has been shown to increase HPA-axis activity after peripheral injection (Barden et al., 1997), elevated corticosterone plasma levels in both wildtype and transgenic mice, demonstrating that HPA axis activity is sensitive to CRH stimulation in both groups. Administration of 8-OH DPAT, however, increased plasma corticosterone levels in wildtype animals, but not in transgenics. Since elevation of plasma ACTH levels induced by 5-HT_{1A} receptor activation may be mediated by 5-HT_{1A} receptors affecting the release of CRH from the PVN (Pan, Gilbert, 1992), it may be speculated that the blunted HPA response seen after 8-OH-DPAT administration in transgenic mice is due to desensitisation of 5-HT_{1A} receptors at the level of the PVN.

Several clinical studies have demonstrated that administration of 5-HT_{1A} agonists results in a decrease in body temperature and an increase in cortisol release in human. Different profiles were found when the responses to 5-HT_{1A} receptor challenge were studied in affective disorders. Thus, patients diagnosed as suffering from unipolar or major depression exhibit significantly decreased cortisol responses to the partial 5-HT_{1A} agonist ipsapirone, while basal cortisol secretion is increased when compared to controls (Lesch et al., 1990; Meltzer, Maes, 1995). Despite altered

HPA responsivity to 5-HT_{1A} challenge, no significant differences were observed in ipsapirone-induced hypothermia (Meltzer, Maes, 1995). This contrasts with data from patients with bipolar depression, who did not differ in cortisol release (Shiah et al., 1998), and patients with mania, who showed increased cortisol release after ipsapirone challenge (Yatham et al., 1999). Hypothermic responses were not different from controls in both patients groups. Patients suffering from obsessive compulsive disorder, on the other hand, showed no differences after 5-HT_{1A} challenge in either parameter (Lesch et al., 1991), whereas attenuation of both thermoregulation and cortisol release was seen in patients with panic disorder (Lesch et al., 1992). Thus, the responses seen in mice overproducing CRH strikingly resemble those observed in depression, and suggest that blocking CRH action may be beneficial in depression and help to restore serotonergic neurotransmission in patients.

In summary, the data indicate that 5-HT_{1A} receptor challenge results in reduced responsiveness in plasma corticosterone levels in mice overproducing CRH, whereas body temperature changes after stress or following 5-HT_{1A} receptor challenge is unaltered. Extrapolating this situation to the human, these data open the possibility that the desensitisation of 5-HT_{1A} receptors seen in the brains of depressed subjects may result from hyperactivity of the CRH system frequently seen in these patients (Steckler and Holsboer 1999).

Chapter 7

Effects of chronic treatment with citalopram on anxiety-related behaviour depends on emotionality state of test subject

Introduction

Data presented in Chapter 4 shows that long-term treatment with citalopram increases some anxiety-related behaviour. This is in contrast to clinical findings where citalopram and other SSRI's are known for their anxiolytic actions after long-term treatment. Here, the hypothesis that SSRI's might only have anxiolytic properties in animals with increased emotionality was addressed further. Therefore, the effects of long-term citalopram treatment on anxiety-related behaviour was compared between mice overproducing CRH and wildtype littermates. Mice overproducing CRH are known to show increased anxiety-related behaviour (Stenzel-Poore et al., 1994; Heinrichs et al., 1997b), although this seems to be task dependent (see Chapter 5). Furthermore, results presented in Chapter 6 show that these animals have an altered response in HPA-axis activity after 8-OH DPAT challenge. Therefore, it was investigated whether chronic citalopram treatment would also normalise the effects of 5-HT_{1A} receptor stimulation on HPA axis activity. This may

be of particular relevance as it has been suggested that anti-anxiety or antidepressant effects of antidepressants might be mediated via enhancement of serotonergic neurotransmission due to desensitisation of 5-HT_{1A} autoreceptors (Albert et al., 1996). Given that 5-HT_{1A} autoreceptor function can be assessed using the 8-OH DPAT induced hypothermia test, it was investigated whether desensitisation of the 5-HT_{1A} autoreceptor function, which occurs after chronic treatment with antidepressants (Bill et al., 1991; Goodwin, 1989), would be affected by long-term overproduction of CRH.

Material and methods

Animals

Individually housed male transgenic mice overproducing CRH (Stenzel-Poore et al., 1992) were compared to age-matched wildtype littermates (n = 10 per group).

Treatment

Animals were treated chronically with citalopram (racemic, kindly donated by Lundbeck, Copenhagen, Denmark) at a dose of 20 mg/kg or vehicle po (as described in detail in Chapter 4). Animals received treatment between 17.00 and 17.30 h.

Behavioural analysis

After being treated for three weeks with citalopram, animals were first tested in the light dark exploration paradigm. Subsequently, locomotor activity was assessed in the open field for 30 min. Then, hole board exploration was assessed for 30 min.

Finally, conditioned fear was assessed 24 h after conditioning. At least 5 days lapsed between tests.

8-OH DPAT induced hyperthermia and HPA-axis activation

Prior to the experiment, animals were weighed and rectal temperature was recorded over 4 seconds. Immediately afterwards, animals received a s.c. injection of either 8-OH-DPAT (0.5 mg/kg dissolved in saline) or saline, and were returned to their homecage for 20 min, followed by another measurement of rectal temperature. Subsequently, animals were anaesthetised with isofluran and decapitated within 15 seconds. Trunk blood was collected for ACTH and corticosterone measurement.

Body weight

The animals were weight before (day 0) and after chronic treatment (day 32).

Statistics

Data were analysed by multiple analyses of variance (MANOVA), followed by post hoc Tukey test, if appropriate.

Results

Light dark exploration

Total distance travelled was decreased in transgenic mice ($F_{1,34} = 42.58$, $p < 0.001$; Figure 7.1A), but was not affected by treatment ($F_{1,34} = 2.63$, $p > 0.05$). Furthermore, a genotype x treatment interaction was found ($F_{1,34} = 5.77$; $p = 0.022$). Post hoc testing revealed that treatment reduced locomotor activity in wildtype animals ($p <$

0.050), but not in transgenic mice. Overall, time spent in the illuminated compartment was decreased in transgenic mice ($F_{1,34} = 7.34$, $p = 0.010$), whilst no overall effect of treatment was found ($F_{1,34} = 2.73$, $p > 0.05$; Figure 7.1B). Furthermore, a genotype x treatment interaction was found ($F_{1,34} = 2.73$, $p = 0.017$). Post hoc testing revealed that time spent in light compartment was decreased by treatment in wildtype animals ($p > 0.010$). A tendency for an increase in time spent in the light compartment was found after treatment in mutants, but failed to reach significance. Likewise, relative distance travelled was decreased in mutants ($F_{1,34} = 7.71$, $p = 0.009$), while no overall treatment effect was found ($F_{1,34} = 1.72$, $p > 0.05$, Figure 7.1C). However, a genotype x treatment interaction was found ($F_{1,34} = 5.69$, $p = 0.023$). Post hoc testing revealed that citalopram treatment tended to reduce relative distance travelled in the light compartment in wildtype animals ($p = 0.052$), whereas treatment seemed to have an opposite effect in mutants, although this failed to reach significance.

Open field

Distance travelled in the open field was reduced in mice overproducing CRH ($F_{1,33} = 23.22$, $p < 0.001$). No overall effect of treatment ($F_{1,33} = 0.50$) or genotype x treatment interaction ($F_{1,33} = 2.31$) were found (both p 's > 0.050 ; Figure 7.2A). Likewise, transgenics made less rearings compared to wildtype animals ($F_{1,33} = 6.23$, $p = 0.018$). Again no effect of treatment was observed (treatment effect $F_{1,33} = 6.22$; genotype x treatment effect $F_{1,33} = 1.34$, both p 's < 0.050 ; Figure 7.2B).

Light Dark Exploration

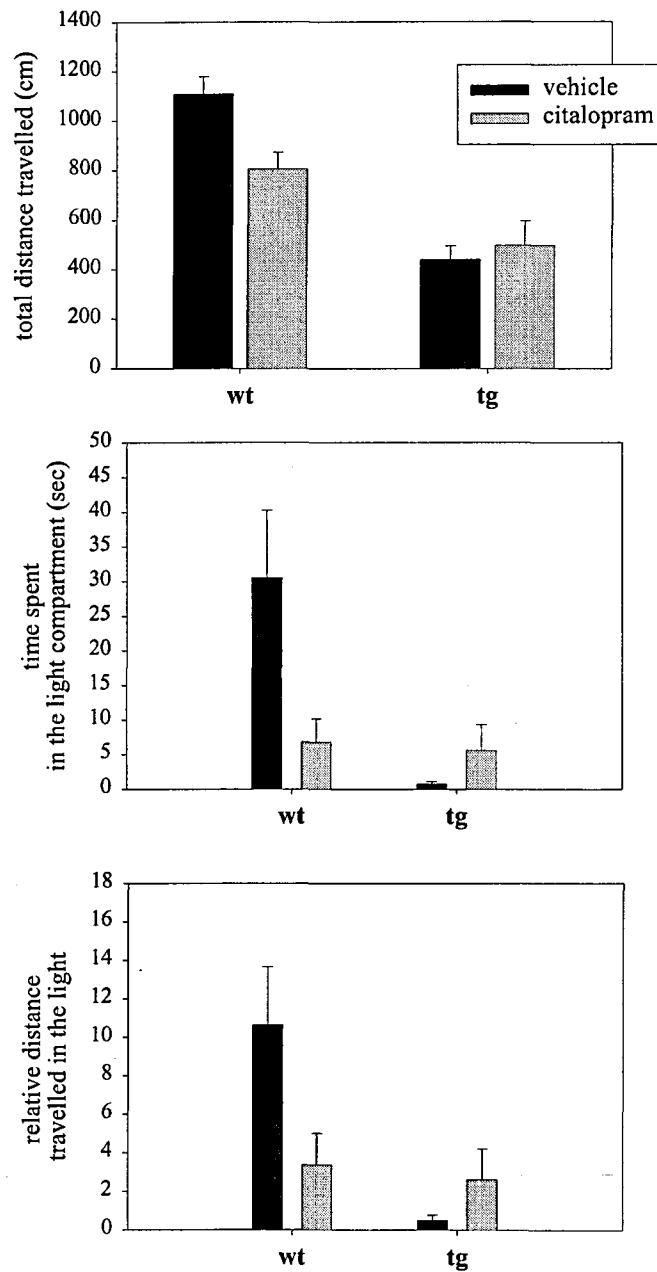


Figure 7. 1 Effects of chronic citalopram treatment in CRH overproducing and wildtype mice on light dark exploration: the total distance travelled over 5 min (A), the time the animal spent in the illuminated part (B), and the relative distance travelled in the light (C) are shown. Data are presented as means, with error bars denoting SEMs.

Hole board exploration

Overall, transgenic mice made less nose pokes compared to wildtype littermates ($F_{1,33} = 5.98$, $p = 0.020$). Treatment did not affect this measure (treatment effect $F_{1,33} = 1.86$; genotype x treatment effect $F_{1,33} = 0.44$ both p 's > 0.050 ; Figure 7.3).

Conditioned fear

The overall response to the cue in term of relative resting time was significantly different between groups ($F_{1,32} = 10.91$, $p < 0.002$ Figure 7.4). Visual inspection of the data revealed that wildtype animals showed an increased relative resting time compared to transgenic mice. No significant session time dependent effects were found (genotype x bin effect $F_{3,96} = 0.58$; treatment x bin effect $F_{3,96} = 1.09$; genotype x treatment x bin $F_{3,96} = 0.48$, all p 's > 0.05) in terms of relative resting times after re-exposure to the cue. Although there was no overall effect of treatment ($F_{1,32} = 0.00$, $p > 0.05$), a significant genotype x treatment interaction was found ($F_{1,32} = 4.46$, $p = 0.043$). Post hoc testing revealed transgenic mice treated with citalopram showed a lower response to re-exposure to the cue compared to wildtype animals treated with citalopram ($p < 0.050$).

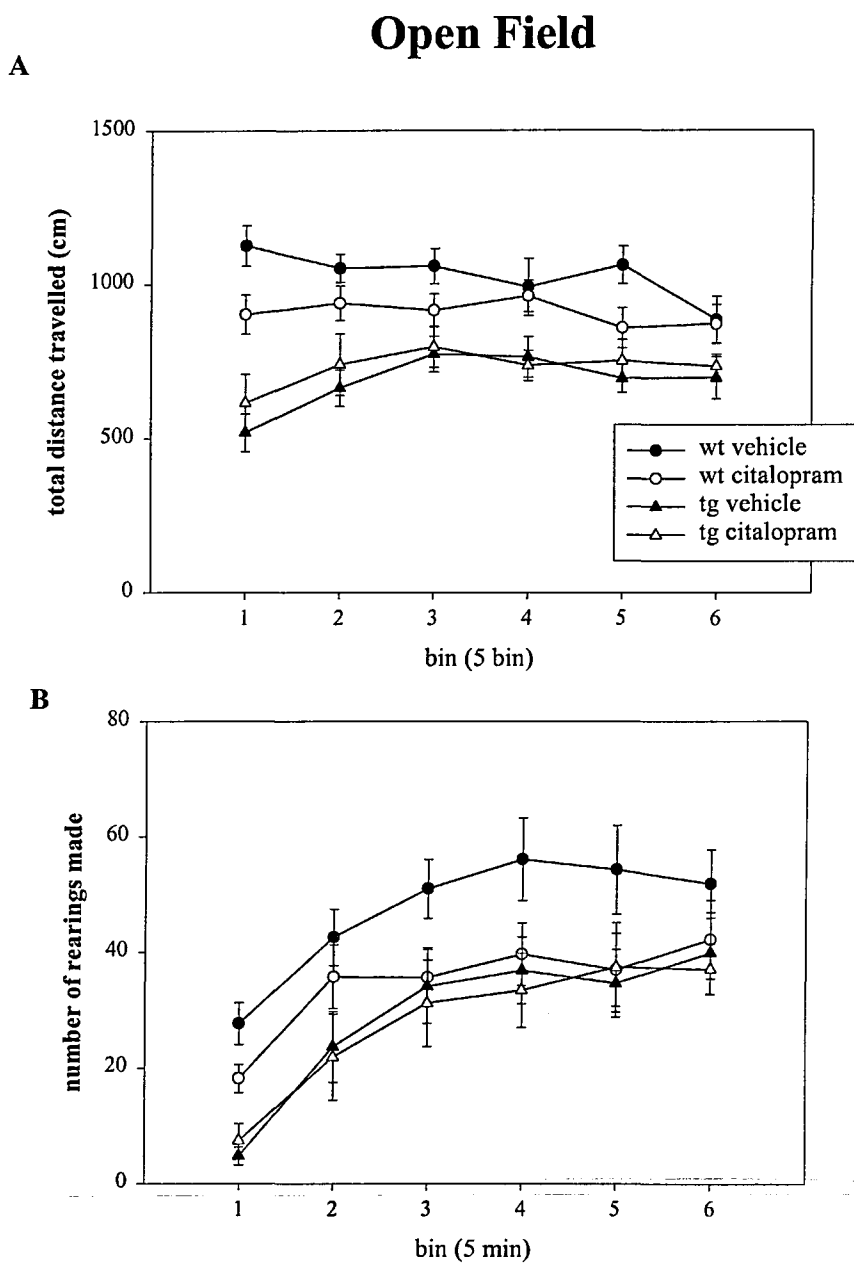


Figure 7.2 Effects of chronic citalopram treatment in CRH overproducing and wildtype mice on behaviour in an open field: the total distance travelled (A) and the number of rearings made (B) are shown. Data are presented as means, with error bars denoting SEMs.

Hole Board

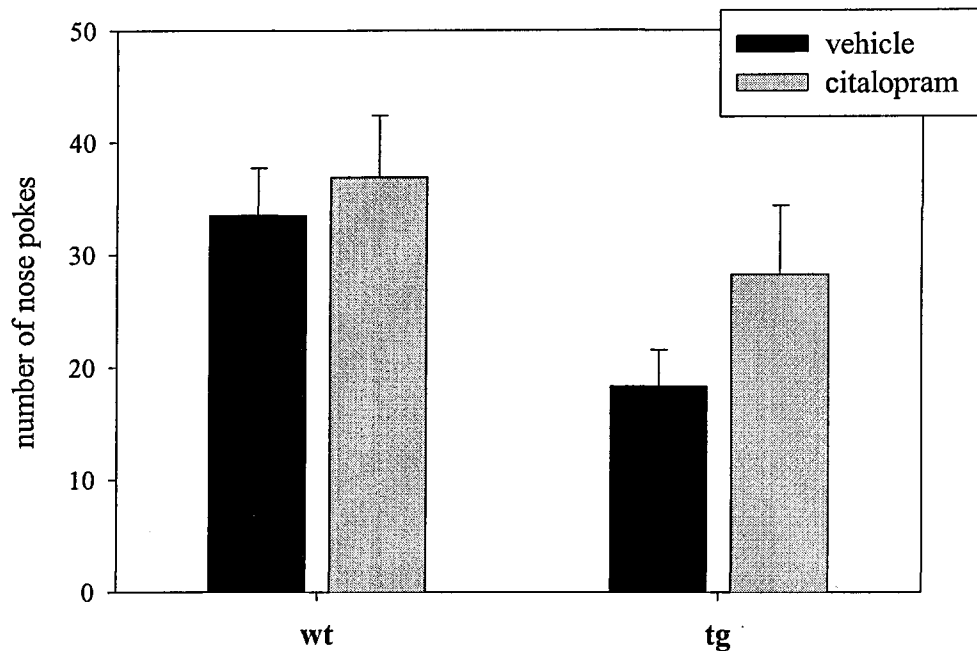


Figure 7.3 Effects of chronic citalopram treatment in CRH overproducing and wildtype mice on hole board exploration: number of nose pokes made during a 30 min session are shown. Data are presented as means, with error bars denoting SEMs.

8-OH DPAT induced hypothermia

Chronic treatment with citalopram attenuated 8-OH DPAT induced hypothermia (treatment x bin effect $F_{1,33} = 5.21$, $p = 0.029$; Figure 7.5). No effect of transgenesis (genotype x bin; $F_{1,33} = 1.20$, $p > 0.05$) or genotype x treatment x bin interaction ($F_{1,33} = 0.43$, $p < 0.050$) was found. Furthermore, transgenic mice had an overall increased body temperature compared to wildtype littermates ($F_{1,33} = 16.23$, $p < 0.001$).

Conditioned Fear

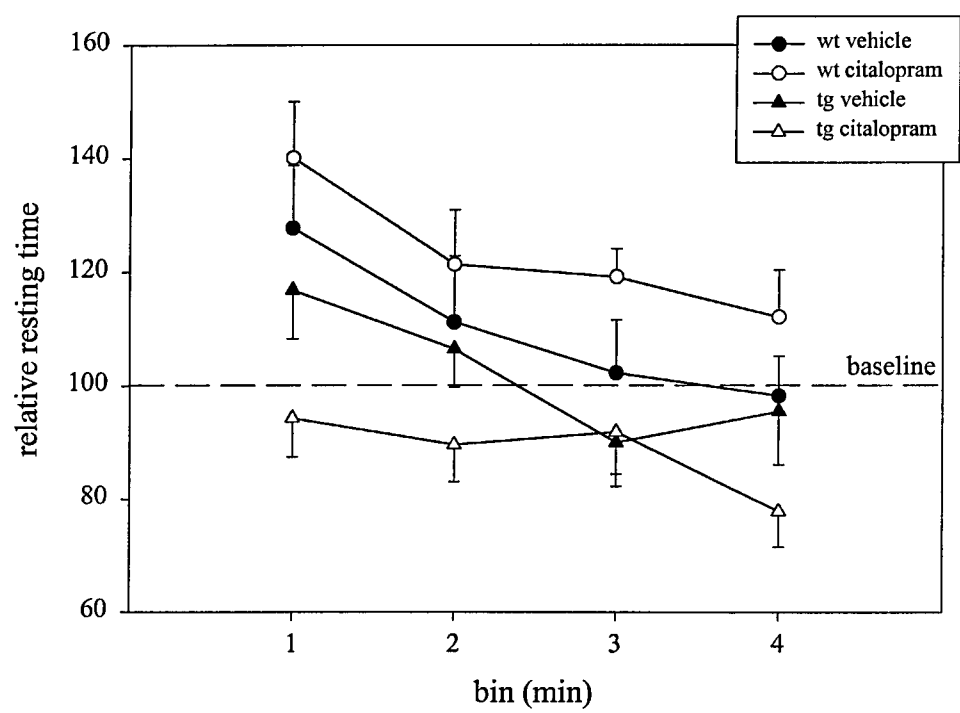


Figure 7.4 Effects of chronic citalopram treatment in CRH overproducing and wildtype mice on conditioned fear: change in the relative time not moving during re-exposure to the light relative to baseline (dotted line) 24 h after conditioning is shown. Data are presented as means, with error bars denoting SEMs.

8-OH DPAT induced hypothermia

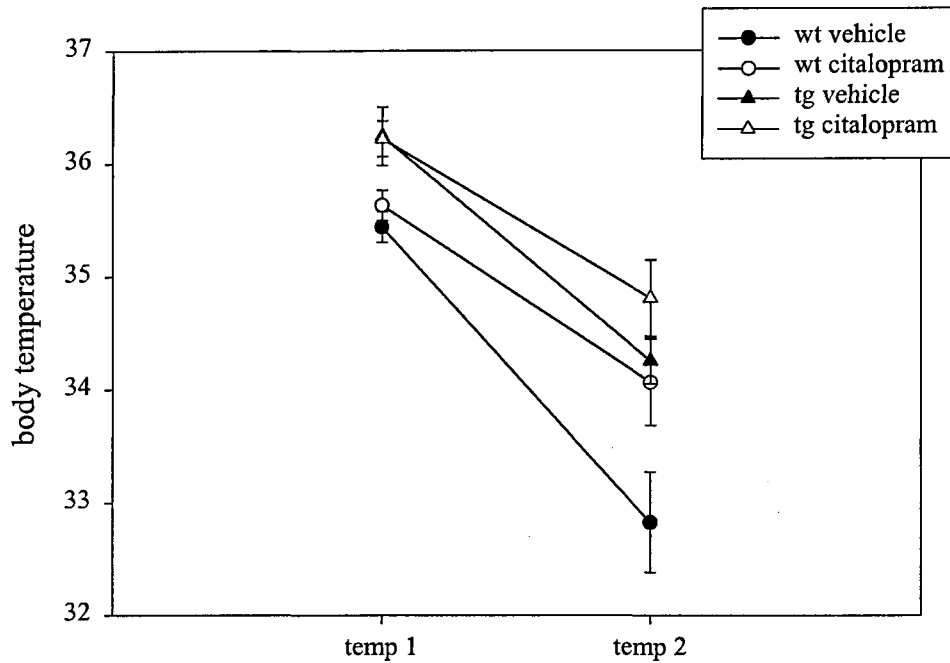


Figure 7.5 Effects of chronic citalopram treatment in CRH overproducing and wildtype mice on body temperature measured under basal conditions (temp 1) and 20 min after treatment (temp 2). wt: wildtype; tg: transgenic. Data are presented as means \pm SEMs.

8-OH DPAT induced HPA-axis activation

8-OH DPAT-induced ACTH release was significantly lower in transgenic animals compared to wildtype mice ($F_{1,30} = 60.18$, $p > 0.001$). No effects of treatment ($F_{1,30} = 0.63$) or a genotype x treatment interaction were found ($F_{1,30} = 0.16$; both p 's > 0.050 ; Figure 7.6A). Likewise, 8-OH DPAT induced corticosterone release was

significantly lower in transgenic animals compared to wildtype mice ($F_{1,32} = 18.25$, $p > 0.001$). No effects of treatment ($F_{1,32} = 2.63$) or genotype x treatment interaction ($F_{1,32} = 0.01$) were found (both p 's > 0.050 ; Figure 6B). Strikingly, however, the corticosterone levels were 1.4 times higher in wildtype mice compared to transgenics, whilst for ACTH the difference was 3.7.

Body weight

Body weight did not differ as a factor of genotype or treatment (genotype effect $F_{1,33} = 0.07$; treatment effect $F_{1,33} = 1.13$; both p 's > 0.050). However, a significant genotype x treatment interaction was found ($F_{1,33} = 4.93$, $p = 0.033$; Figure 7.7). Post hoc testing revealed that mutant mice did not differ in body weight when treated with vehicle, but CRH overexpressing mice treated chronically with citalopram had significantly lower body weights than wildtype littermates which received the same treatment.

Discussion

In this study, it was demonstrated that effects of long-term treatment with citalopram has opposite effects on anxiety-related behaviour in mice overproducing CRH compared to wildtype littermates. Thus, in the light dark exploration task, citalopram increases anxiety-related behaviour in wildtype animals, whereas in transgenics, which already displayed increased anxiety-related behaviour in this task under basal conditions, it decreased anxiety-related behaviour. Likewise, citalopram treatment induced opposite effects on anxiety-related behaviour measured in the conditioned fear paradigm, although transgenic animals did not display significant altered respon-

8-OH DPAT induced HPA-axis activation

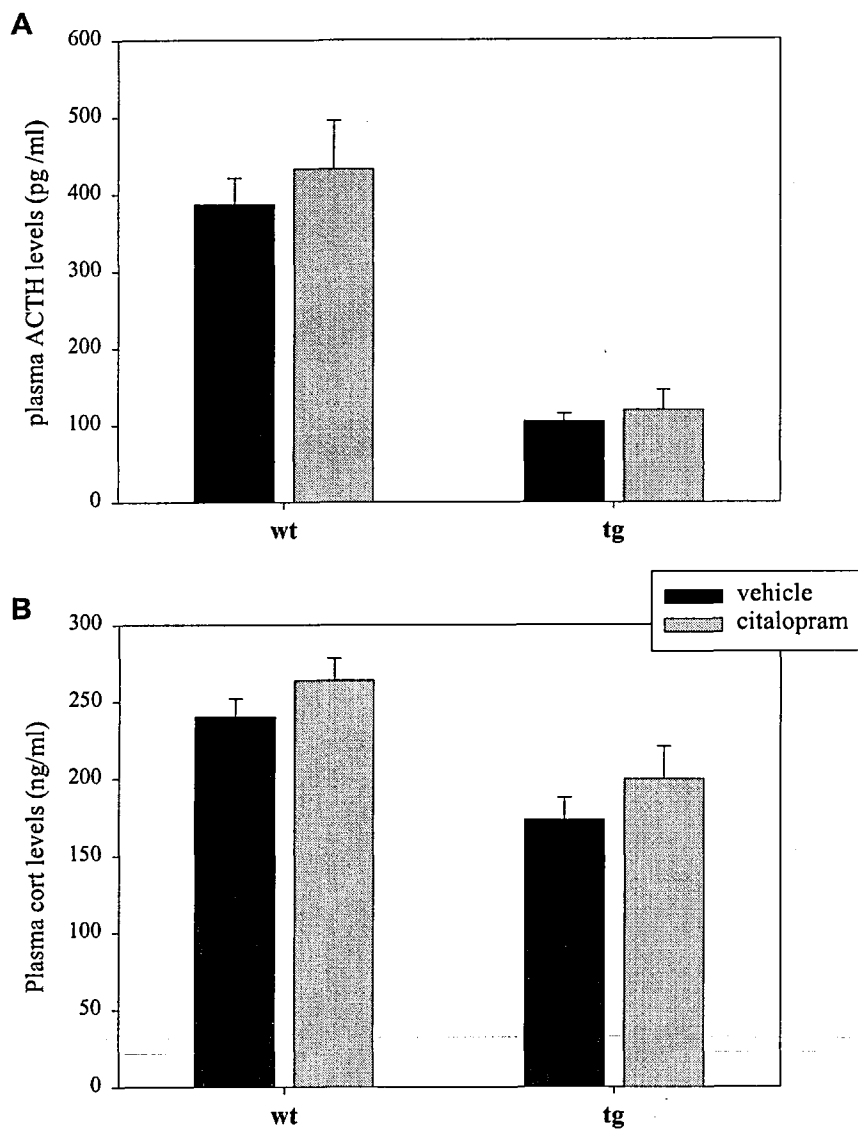


Figure 7.6 Effects of chronic citalopram treatment in CRH overproducing and wildtype mice on plasma ACTH (A), and corticosterone (B) levels, 20 min after injection of 8-OH-DPAT (0.5 mg/kg). wt: wildtype; tg: transgenic. Data are presented as means \pm SEMs.

Body Weight

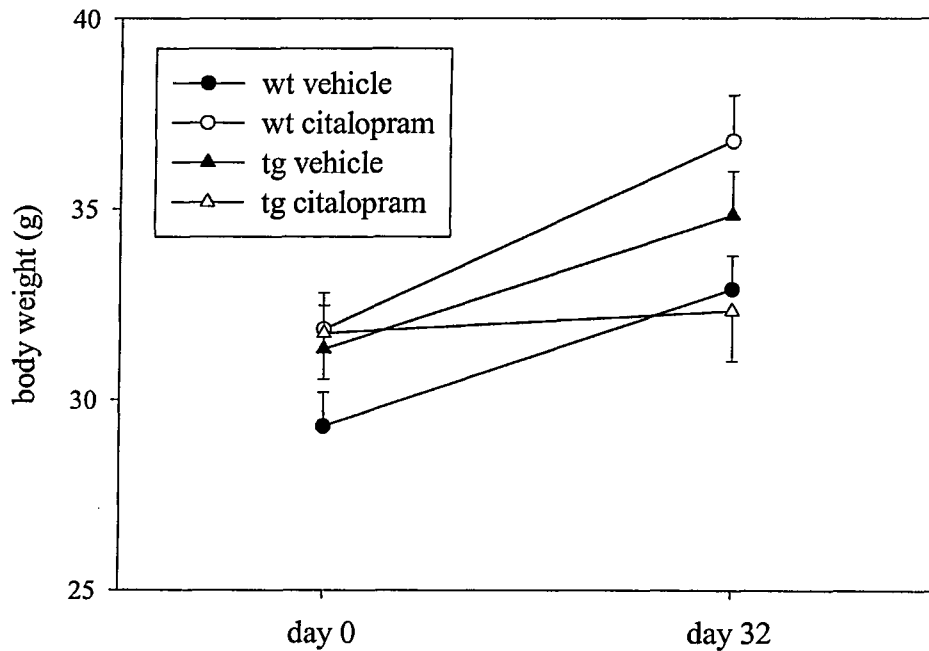


Figure 7.7 Effects of chronic citalopram treatment in CRH overproducing and wildtype mice on body weight before and 32 days after beginning of treatment. wt: wildtype; tg: transgenic. Data are presented as means \pm SEMs.

sivity to the CS without treatment in this task. The results from the light dark exploration are in line with a recently reported study by Keeny and Hogg (1999). The authors showed that chronic treatment with citalopram reduces anxiety-related behaviour in light dark exploration after mice having been chronically socially defeated, whereas it was without effect in controls.

Citalopram did not affect locomotor activity in terms of distance travelled or number of rearings made in the open field, and, as already demonstrated in Chapter 5, transgenics were hypoactive. Furthermore, transgenics displayed overall reduced

exploratory behaviour in the hole board paradigm, whereas in Chapter 5 the reduced exploratory behaviour was limited to the early testing session. Citalopram tended to increase exploratory behaviour, as it did in Swiss Webster mice (Chapter 4).

Citalopram treatment resulted in desensitisation of 5-HT_{1A} autoreceptors, as indicated by the attenuated hypothermic response to 8-OH DPAT administration. This effect was independent of genotype. Such a desensitisation is believed to result in an increase of serotonergic neurotransmission. In animal paradigms, it is often seen that reduction of serotonergic neurotransmission is accompanied by reduction of anxiety-related behaviour (Handley, 1995). For example, exposure of a rat to an elevated plus maze increases hippocampal serotonin. Pre-treatment with diazepam prevents this increase and reduces anxiety-related behaviour on the maze (Wright et al., 1992). This suggests that an increase in serotonergic neurotransmission may result in an increase in anxiety. However, in depression, it is believed that serotonergic function is reduced (Maes and Meltzer, 1995). Furthermore, increased anxiety is often observed in depressed patient. Besides, treatment with SSRI's is effective in depression, but also in several anxiety disorders. This suggests that both increased or decreased serotonergic may result in increased anxiety. This is in line with the hypothesis that imbalanced serotonergic neurotransmission may contribute to both depression and anxiety disorders (Eison, 1990).

Changes found in behaviour and neuroendocrinology in CRH overproducing mice bears some similarities with symptoms of depression, such as increased anxiety. Furthermore, it has been demonstrated that long-term i.c.v. administration of CRH decreases serotonin release in the hippocampus after stress exposure in rats

(Linthorst et al., 1997). Therefore, it is possible that chronic CRH excess in these mice also results in reduced serotonergic function. This in turn opens the possibility that dissociative effects of citalopram treatment on anxiety-related behaviour are related to increasing serotonergic transmission in CRH overexpressing animals which would be beneficial, comparable to the situation seen in depressive patients. In contrast, citalopram may be anxiogenic due to imbalancing the serotonergic system in wildtype mice.

Alternatively, the opposite effects of treatment on anxiety-related related behaviour could also be due to an CRH induced difference in sensitivity or density of 5-HT receptors other than the 5-HT_{1A} autoreceptor. If this occurs, increase in serotonergic transmission would augment different postsynaptic 5-HT receptors, which play distinct roles in anxiety-related behaviour (Griebel 1995). A likely candidate is the postsynaptic 5-HT_{1A} receptor, as CRH transgenics are less susceptible to 8-OH DPAT induced HPA-axis activation, a phenomenon which is, at least in part, mediated via post-synaptic 5-HT_{1A} receptors (Chapter 6). However, other subtypes cannot be excluded.

However, it is also possible that the effects of SSRI's on anxiety-related behaviour are secondary to it's effect on serotonin modulation. For example, it has been hypothesised that antidepressants, rather than acting through a specific pharmacological mechanism, may trigger a long cascade of events which then converge to act through a final common pathway (Delgado et al., 1999). Furthermore, it has been shown that tianeptine, a 5-HT re-uptake enhancer, and fluoxetine, a 5-HT re-uptake blocker, both exhibit good efficacy for treatment of

depressive disorders, which also supports the idea that the primary effect of antidepressant-induced changes on uptake transport is remote from the therapeutically relevant action (Loo et al., 1999). Taken together, this suggests that therapeutic effects of treatment with SSRI's may be due to secondary effect and not due to enhancement of serotonergic neurotransmission per se. Further experiments are needed in order to draw conclusions which system(s) may underlie the alterations in anxiety-related behaviour following long-term CRH excess.

Interestingly, diminished HPA-axis activation after 5-HT_{1A} receptor challenge is known in depressives, which also occurs in CRH overexpressing mice. This may open the possibility that citalopram affects anxiety-related behaviour via HPA-axis normalisation. However, chronic treatment affected anxiety-related behaviour and the serotonergic system, whereas HPA-axis activation after 8-OH DPAT administration remained unaffected, suggesting that the anxiogenic effects of long-term CRH excess are independent from HPA-axis activity.

The citalopram-induced increase in body weight observed in Chapter 4 was not seen in this study. In fact, all groups gained body weight during the experiment, except for the transgenic mice that received citalopram. Clinical studies have indicated that the effects of SSRI treatment on body weight depend on the neurobiology of the depressive illness (Harvey and Bouwer, 2000). The results from this study suggest that the different effects of SSRI's on body weight may depend on the activity of the CRH system.

Long-term alterations in the corticotropin-releasing hormone system: effects on emotional function and attention

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Chapter 8

Effects of overproducing CRH on attention in mice

Introduction

In the previous chapters it was demonstrated that long-term CRH excess increases some aspects of anxiety-related behaviour (Chapter 5, 7). Furthermore, this type of behaviour could be diminished by chronic treatment with SSRI's (Chapter 7). Thus, the phenotype of CRH overexpressing mice has resemblance with that seen in depressive patients. In addition, the attenuated HPA-axis response to 5-HT_{1A} challenge, with the unaltered response in body temperature, is also comparable (Chapter 6). This, together with the finding that the CRH system is hyperactive in depressive illness, suggests that CRH excess may be the cause of symptoms seen in depression.

Apart of its known effects on anxiety, CRH is also known to mediate arousal. Arousal can be defined as a state which optimises the processing of sensory stimuli in the cerebral cortex (Hebb, 1955) and is therefore related to attentional function

(Robbins, Everitt, 1994). This in turn suggests that CRH plays a more general role than just to mediate responses to aversive events, but maintains attention towards events or cues of biological significance, regardless of the nature of the reinforcer (Merali et al., 1998).

Patients suffering from major depression display attentional deficits (Mialet et al., 1996; Rief and Hermanutz, 1996; Lemelin et al., 1997; Williams et al., 2000). Whether disruption of attentional processes is due to alteration in CRH activity is unknown. CRH receptors, in particular the CRHR1, occur in brain areas that play a role in attentional processes (see Chapter 1). Furthermore, high degrees of co-localisation of choline-acetyltransferase and CRH receptor-like immunoreactivity have been observed in both the murine basal forebrain and brainstem (Sauvage and Steckler, 2001). The prefrontal cortex (Muir et al., 1996), the superior colliculi (Overton and Dean, 1988) and the cholinergic basal forebrain (Muir et al., 1992a; 1994; Robbins et al., 1989) have been implicated in the mediation of different types of attention, and the cholinergic brainstem nuclei have been also demonstrated to control arousal and attentional functions (García-Rill, 1991; Steckler et al., 1994). Moreover, in rats, intracerebroventricular (i.c.v.) CRH produces stimulation of Fos expression within the basal forebrain and brainstem nuclei (Bittencourt and Sawchenko, 2000), and ICV, but not peripheral, injections of CRH increase hippocampal ACh release (Day et al., 1998a, 1998b). Taken together, these observations support the idea that CRH might play a prominent role in the modulation of attentional processes.

In this chapter, the possibility that CRH affects attentional processes is addressed using transgenic mice overproducing CRH. CRH overexpressing transgenics have been reported to be impaired in learning forced-choice alternation and water maze place navigation tasks. Paradoxically, however, this place navigation deficit was attenuated by administration of the benzodiazepine chlordiazepoxide (CDP), which by itself is known for its amnesic properties. This in turn suggests that heightened anxiety or overarousal possibly confounds the navigation deficit seen in transgenic animals (Heinrichs et al., 1996).

If it is true that these transgenic animals suffer from overarousal, impairments to detect relevant stimuli and to process them would be expected. To test this hypothesis more directly, we trained transgenic mice overexpressing CRH and wildtype littermates on a murine version of a 5-choice serial reaction time task (5-CSRTT).

Material and methods

Animals

15 heterozygous male CRH overexpressing mice and nine male wildtype littermates, bred and reared in the animal house of the Max Planck Institute (for more detail see Chapter 5) were used. Animals were housed individually and maintained on a 12:12 h light/dark cycle (lights on at 06:00 hours) with *ad lib* water throughout. They were maintained at 85-90% of their free feeding weight, starting one week prior to the beginning of the experiment by restricting the laboratory chow access to 3.0-3.5 g /

day / mouse (depending on the number of pellets eaten during testing). All experiments were conducted during the light phase of the cycle.

Behavioural procedure

Animals were trained as described in detail in Chapter 2. In brief, animals were habituated to the testing apparatus until they reliably ate all the pellets presented (2 per hole). Next, mice were trained on the autoshaping procedure. Seven sessions were scheduled during this experimental stage. Then, the animals were trained to acquire a simple visual discrimination (see protocol Chapter 2). After stable performance was reached, the stimulus duration was gradually reduced (i.e., 8 s, 4 s, 2 s, 1 s, 0.5 s), with 5 sessions per stimulus duration.

Cued detection

Once animals reached stable performance, predictability of the stimulus was enhanced by introduction of a pre-sound (1.2 kHz, 76 dB), presented during the 500 ms preceding light stimulus onset (cued detection). Eight sessions were scheduled using this procedure, followed by an additional nine sessions without tone cue.

Manipulations of basic task parameters

Next, a series of manipulations of the basic task were made which were designed to increase the attentional load. First, the ITI durations were gradually reduced (5.0, 2.5, 1.0 seconds) after which they were increased (5.0, 7.5, 10 seconds). This is believed to increase the attentional load by disrupting the temporal predictability of the stimulus onset. During this phase, ITI respondings were not analysed.

Drug treatment

Subsequently, drug treatment commenced. Animals were first treated with diazepam (Sigma Chemicals, Deisenhofen, Germany), dissolved in saline plus 3 % tween 20 (0.0, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0, 15.0 mg/kg i.p., 20 min prior to test). At least two days lapsed between drug testing in order to allow for sufficient wash-out and recovery of the animals. This was followed by treatment with various doses of the acetylcholinesterase inhibitor physostigmine (0.0, 0.05, 0.10, 0.25, 0.50 mg/kg i.p., dissolved in saline, 20 min prior to test;). Subsequently, the effect of acute administration of the CRHR1 antagonist R121919 (using saline plus 3 % tween 20 as vehicle) was studied with various doses (0.0, 10.0, 20.0, 40.0 mg/kg i.p., 20 min prior to test). Finally, animals were treated with the muscarinic antagonist scopolamine (0.0, 0.1, 0.5, 1.0 mg/kg i.p., 20 min prior to test; dissolved in saline). All treatments were given in ascending order.

Results

Autoshaping procedure

All wildtype animals completed the 20 trials within the time limits of 20 min from the first session onwards, while transgenic mice responded at slightly lower level (average 19.01 trials completed per session; genotype effect $F_{1,22} = 4.57$, $p = 0.044$) (Figure 8.1A). However, there was no effect of session (session effect $F_{6,132} = 0.55$, $p > 0.05$) or interaction between session and genotype (genotype x session effect $F_{6,132} = 0.55$, $p > 0.05$), indicating that both groups of animals maintained their level of responding. This reduction in responsivity in transgenic mice was also reflected in

the average number of nose-pokes made during the ITI (genotype effect $F_{1,21} = 12.08$, $p = 0.002$). Again no effects of session (session effect $F_{6,126} = 0.98$, $p > 0.05$) and no interaction (genotype \times session effect $F_{6,126} = 1.75$, $p > 0.05$) were observed on this measure (Figure 8.1B). Consequently, both the time needed by transgenic animals to complete a trial (genotype effect $F_{1,21} = 16.04$, $p = 0.001$; session effect $F_{6,126} = 1.44$; genotype \times session effect $F_{6,126} = 0.87$, both p 's > 0.050) and the average response latency (genotype effect $F_{1,21} = 42.72$, $p < 0.001$; session effect $F_{6,126} = 2.06$; genotype \times session effect $F_{6,126} = 2.03$, both p 's > 0.050) were increased relative to wildtype performance (Figures 8.1C, D). Moreover, transgenic mice completed less trials prior to the end of the limited hold (i.e., within the 5 s prior to pellet delivery) than wildtype mice (genotype effect $F_{1,21} = 14.04$, $p = 0.001$), and both a session (session effect $F_{6,126} = 4.05$, $p = 0.005$) and genotype \times session interaction (genotype \times session effect $F_{6,126} = 7.48$, $p < 0.001$) were observed for this measure, with groups showing increasing differences during late sessions. Thus, groups differed in that wildtype mice, but not transgenic mice, started to associate the light stimulus with reinforcement towards the end of the autoshaping session (Figure 8.1E), although both groups clearly had associated the response holes with reward, as indicated by the response level maintained over sessions.

Acquisition of a simple visual discrimination

During the initial stage of visual discrimination, the attentional load was kept to a minimum by presentation of a salient stimulus (an illuminated hole in an otherwise dark operant chamber) of long duration (maximally 8 s). Both groups of animals responded above chance from the first session onwards, indicating that they had already learned to associate the light stimulus with reinforcement during

autoshaping, and continued to improve percentage correct responses made over sessions (session effect $F_{8,144} = 9.76$, $p < 0.001$) (Figure 8.2B). Overall, however, transgenic mice performed below wildtype level (genotype effect $F_{1,18} = 8.24$, $p = 0.010$), while no genotype \times session interaction was observed (genotype \times session effect $F_{8,144} = 0.53$, $p > 0.05$), indicating a parallel shift (Figure 8.2B). At the same time, transgenic mice made more errors of omission (genotype effect $F_{1,19} = 26.28$, $p < 0.001$; session \times genotype effect $F_{8,152} = 1.87$, $p > 0.05$) and earned less reinforcers (genotype effect $F_{1,20} = 47.75$, $p < 0.001$), but this effect declined with time on task (session \times genotype effect $F_{8,160} = 2.93$, $p = 0.030$) (Figures 8.2A, C). Moreover, the number of ITI responses made was decreased in CRH overexpressors (genotype effect $F_{1,20} = 36.63$, $p < 0.001$; session \times genotype effect $F_{8,160} = 2.54$, $p = 0.037$) (Figure 8.2D). There was a tendency for transgenic animals to have longer correct response latencies (genotype effect $F_{1,17} = 4.17$, $p = 0.057$; session \times genotype effect $F_{8,136} = 1.17$, $p > 0.05$), and an interaction was seen for the incorrect response latency measure (genotype effect $F_{1,18} = 0.29$, $p > 0.05$; session \times genotype effect $F_{8,144} = 3.46$, $p = 0.007$) (Figures 8.2E, F). Genotypes differed during the initial two sessions but not thereafter in incorrect response latency. In addition, biased responding was increased in transgenic animals (genotype effect $F_{1,17} = 6.30$, $p = 0.022$; session \times genotype effect $F_{8,136} = 0.91$, $p > 0.05$) (Figure 2G).

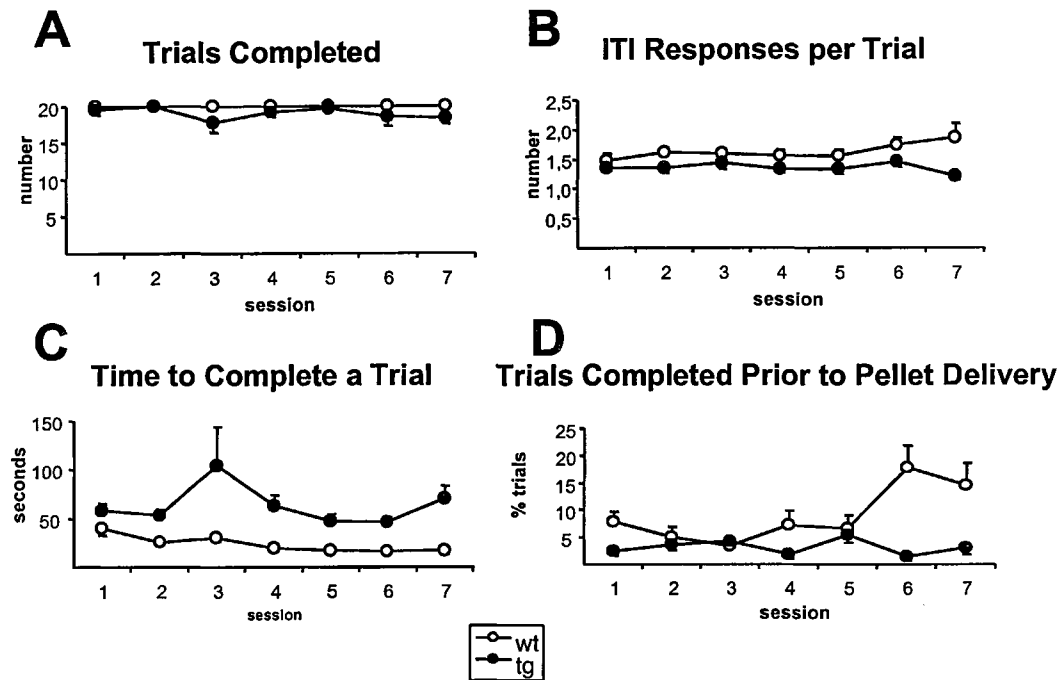


Figure 8.1 Effects of overproduction of CRH on performance during autoshaping procedure. Animals were tested for 7 successive sessions. (wt) wildtype mice, (tg) CRH overexpressing mice. (A) Number of trials completed, (B) ITI responses per trial, (C) time to complete a trial, (D) average response latency and (E) trials completed prior to pellet delivery. Data are presented as means, with error bars denoting SEMs.

Reduction of stimulus duration

Decreasing the stimulus duration led to a decrease in percentage correct responses (stimulus duration effect $F_{3,60} = 67.58$, $p < 0.001$) and decreased the number of reinforcers earned (stimulus duration effect $F_{3,60} = 4.70$, $p = 0.028$) (Figures 8.3A, B). Despite an increasing attentional load, errors of omission also declined with decreasing stimulus duration (stimulus duration effect $F_{3,60} = 36.23$, $p < 0.001$) (Figures 8.3C).

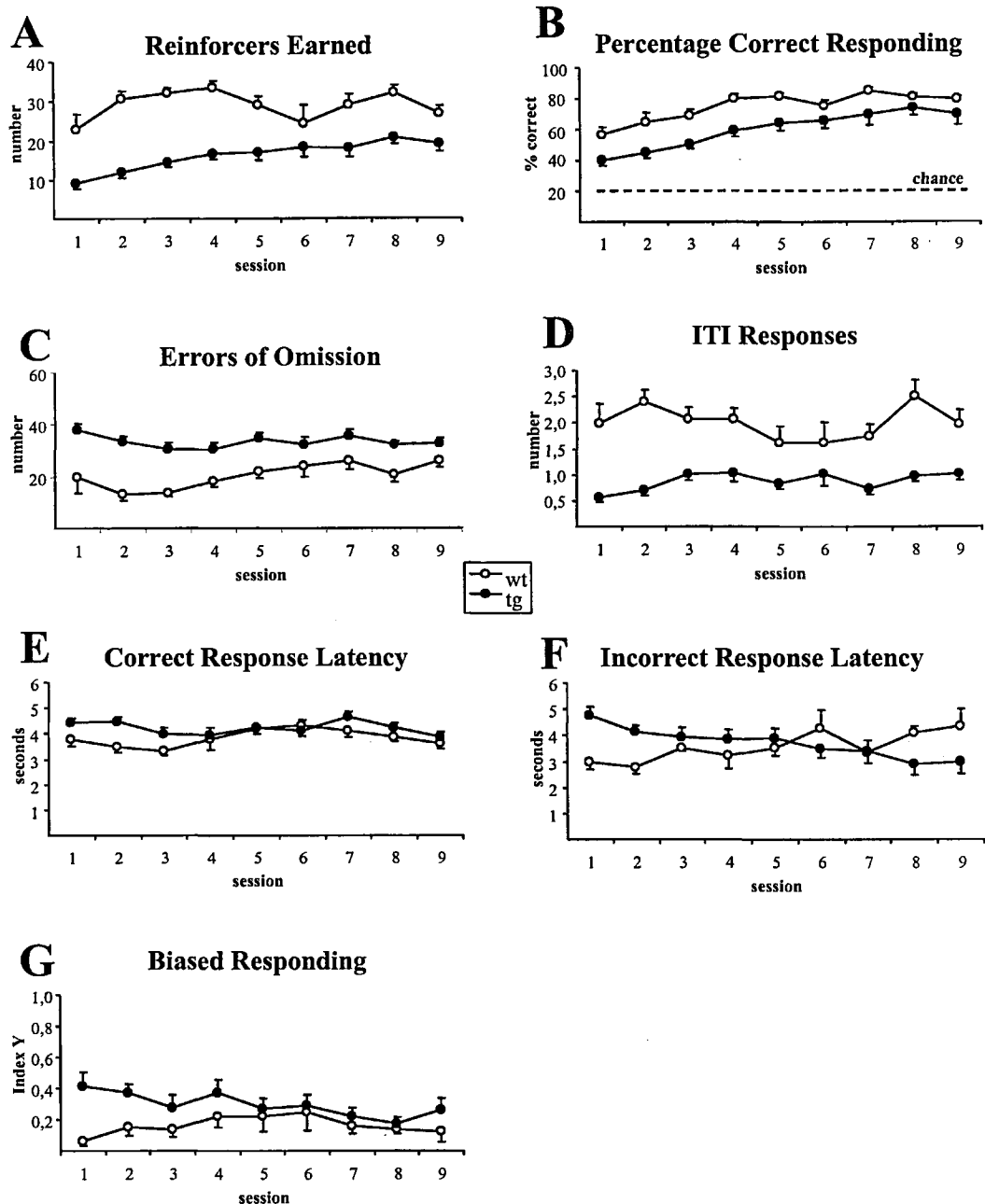


Figure 8.2 Effects of overproduction of CRH on five-choice simultaneous discrimination. Animals were tested for 9 successive sessions, with a stimulus duration of 8 sec. (wt) wildtype mice, (tg) CHR overexpressing mice. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

The number of responses made during the ITI remained unaffected by stimulus duration (stimulus duration effect $F_{3,60} = 2.22$, $p > 0.05$), while both correct and incorrect latencies decreased with decreasing stimulus duration (stimulus duration effect $F_{3,60} = 155.15$, $p < 0.001$ and $F_{3,60} = 27.93$, $p < 0.001$, respectively) (Figures 8.3E, F). Moreover, biased responding increased as a consequence of decreasing stimulus duration (stimulus duration effect $F_{3,60} = 4.26$, $p < 0.011$) (Figure 8.3G).

Although accurate responding remained unaffected by genotype during this experimental stage (genotype effect $F_{1,20} = 1.50$; genotype \times stimulus duration effect $F_{3,60} = 0.77$; genotype \times stimulus duration \times session effect $F_{12,240} = 0.43$, all p 's > 0.050) (Figure 8.3B), transgenic mice continued to earn less reinforcers (genotype effect $F_{1,20} = 21.78$, $p < 0.001$; genotype \times stimulus duration effect $F_{3,60} = 1.45$; genotype \times stimulus duration \times session effect $F_{12,240} = 2.17$, both p 's $= 0.062$) and made more errors of omission (genotype effect $F_{1,20} = 42.63$, $p < 0.001$; genotype \times stimulus duration effect $F_{3,60} = 1.06$; genotype \times stimulus duration \times session effect $F_{12,240} = 0.81$, all p 's > 0.050) (Figures 8.3A, C). CRH overexpressors continued to make less ITI responses (genotype effect $F_{1,20} = 23.47$, $p < 0.001$; genotype \times stimulus duration effect $F_{3,60} = 2.22$; genotype \times stimulus duration \times session effect $F_{12,240} = 1.59$, both p 's > 0.050) (Figure 8.3D). However, the effect of genotype on correct response latencies now gained significance (genotype effect $F_{1,20} = 6.62$, $p = 0.018$; genotype \times stimulus duration effect $F_{3,60} = 1.45$; genotype \times stimulus duration \times session effect $F_{12,240} = 0.78$, both p 's > 0.050), while no effect was seen on incorrect response latencies (genotype effect $F_{1,16} = 0.71$; genotype \times stimulus duration effect $F_{3,48} = 1.15$; genotype \times stimulus duration \times session effect $F_{12,192} = 0.50$, all p 's > 0.050) (Figures 8.3E, F). Biased responding also remained increased

in transgenics (genotype effect $F_{1,20} = 4.54$, $p = 0.046$; genotype \times stimulus duration effect $F_{3,60} = 1.64$, $p > 0.05$; genotype \times stimulus duration \times session effect $F_{12,240} = 0.83$, $p > 0.05$) (Figures 8.3G).

Extended testing with short stimulus durations

When animals were tested for an extended period of 17 sessions with shortest stimulus duration (0.5 s), ANOVA revealed a mild but significant impairment in percentage correct responding (genotype effect $F_{1,20} = 12.91$, $p = 0.002$; genotype \times session effect $F_{16,320} = 1.11$, $p > 0.05$) (Table 8.1). Transgenics continued to earn less reinforcers (genotype effect $F_{1,20} = 130.39$, $p < 0.001$) and an interaction between session and genotype was observed for this measure (genotype \times session effect $F_{16,320} = 2.69$, $p = 0.016$). Post-hoc analysis indicated that groups differed during all sessions except for the second session with short stimulus duration. Likewise, errors of omission remained increased in transgenics (genotype effect $F_{1,20} = 52.38$, $p < 0.001$; genotype \times session effect $F_{16,320} = 0.73$, $p > 0.05$), while ITI responses remained unaffected by transgenesis (genotype effect $F_{1,20} = 0.16$; genotype \times session effect $F_{16,320} = 0.71$, both p 's > 0.050) (Table 8.1).

Transgenic mice also continued to have longer correct response latencies (genotype effect $F_{1,20} = 4.61$, $p = 0.044$; genotype \times session effect $F_{16,320} = 0.70$, $p > 0.05$), while incorrect latencies did not differ between groups (genotype effect $F_{1,20} = 1.21$; genotype \times session effect $F_{16,320} = 0.62$, both p 's > 0.050). Furthermore, overexpressors show increased biased responding (genotype effect $F_{1,20} = 11.41$, $p = 0.003$; genotype \times session effect $F_{16,320} = 1.13$, $p > 0.05$) (Table 8.1).

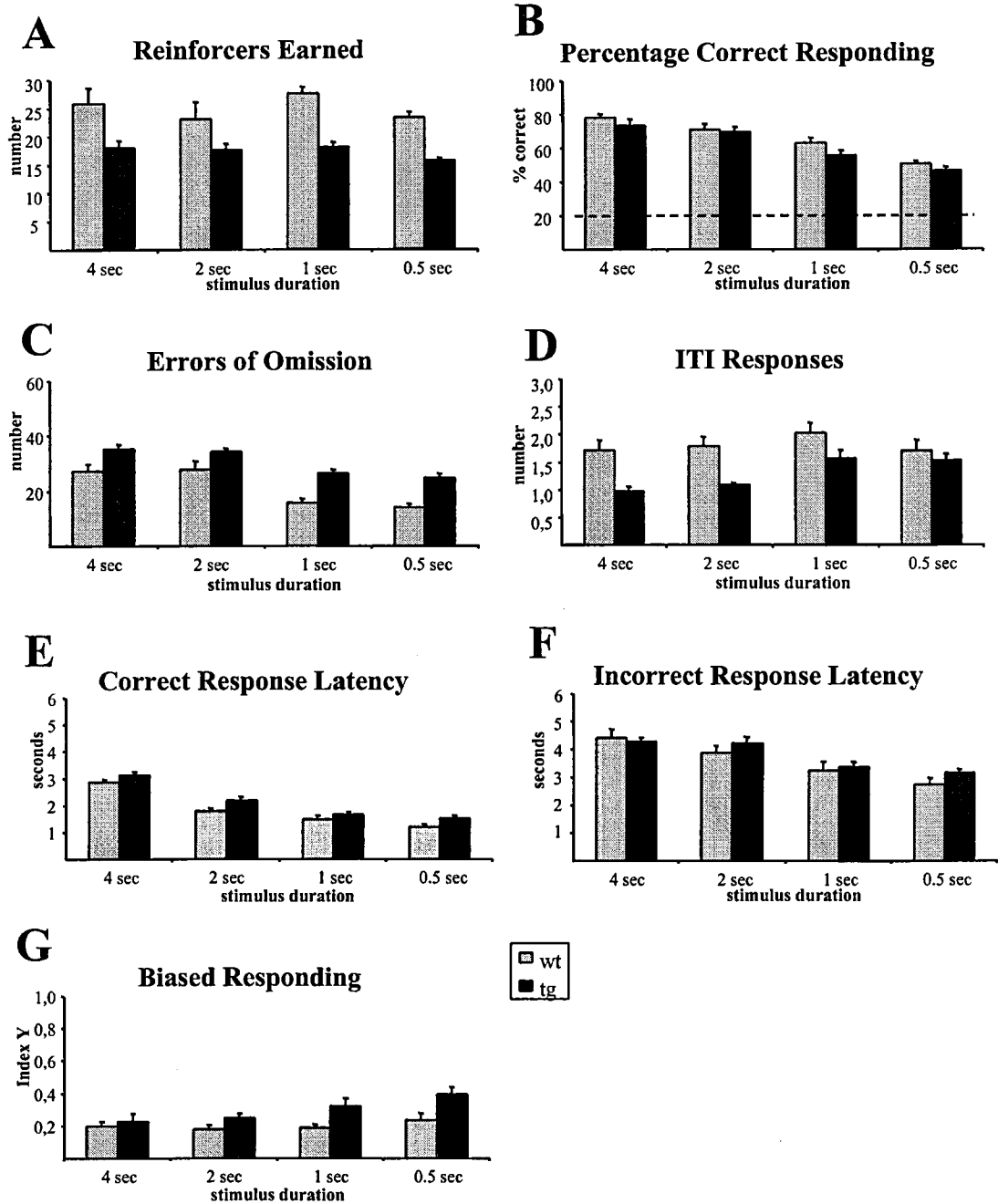


Figure 8.3 Effects of gradual reduction of the stimulus duration on 5-CSRTT performance in mice overproducing CRH. Animals were tested for 5 sessions for each stimulus duration. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

Table 8.1 5-CSRRT performance in CRH overexpressing transgenic mice under basal conditions and following cued detection (data are expressed as means \pm SEMs).Significant differences between groups are indicated by grey shadings.

Measure	Baseline performance (17 sessions)	Cued detection (8 sessions)	Baseline performance (9 sessions)
no. reinforcers earned	wt: 25.21 \pm 0.67	wt: 22.94 \pm 1.70	wt: 23.81 \pm 1.32
	tg: 15.66 \pm 0.50	tg: 14.13 \pm 1.28	tg: 16.28 \pm 1.00
% correct responses	wt: 55.32 \pm 1.85	wt: 61.55 \pm 2.94	wt: 61.21 \pm 2.32
	tg: 46.99 \pm 1.40	tg: 48.75 \pm 2.22	tg: 55.01 \pm 1.75
errors of omission	wt: 14.10 \pm 1.29	wt: 22.92 \pm 2.79	wt: 21.40 \pm 1.97
	tg: 25.69 \pm 0.98	tg: 29.86 \pm 2.11	tg: 29.69 \pm 1.49
ITI responses	wt: 1.50 \pm 0.12	wt: 1.15 \pm 0.11	wt: 1.24 \pm 0.11
	tg: 1.44 \pm 0.09	tg: 1.09 \pm 0.09	tg: 1.19 \pm 0.08
corr. resp. latency (sec)	wt: 1.18 \pm 0.15	wt: 1.10 \pm 0.15	wt: 1.27 \pm 0.14
	tg: 1.58 \pm 0.11	tg: 1.62 \pm 0.11	tg: 1.55 \pm 0.11
inc. resp. latency (sec)	wt: 2.89 \pm 0.18	wt: 2.49 \pm 0.30	wt: 3.30 \pm 0.17
	tg: 3.15 \pm 0.15	tg: 3.12 \pm 0.23	tg: 3.36 \pm 0.13
bias (Index Y)	wt: 0.24 \pm 0.04	wt: 0.22 \pm 0.04	wt: 0.25 \pm 0.03
	tg: 0.39 \pm 0.03	tg: 0.39 \pm 0.03	tg: 0.32 \pm 0.02

No.: number; Corr. Resp. Lat.: correct response latency; Incorr. Resp. Lat.: incorrect response latency.

Cued detection

When light stimulus presentation was preceded by a brief tone stimulus, performance of the animals remained essentially unchanged. Thus, wildtype mice continued to earn more reinforcers (genotype effect $F_{1,20} = 17.13$, $p = 0.001$; genotype \times session effect $F_{7,140} = 1.25$, $p > 0.05$), performed more accurate than transgenics (genotype effect $F_{1,20} = 12.05$, $p = 0.002$; genotype \times session effect $F_{7,140} = 0.29$, $p > 0.05$), had shorter correct response latencies (genotype effect $F_{1,20} = 7.97$, $p = 0.011$; genotype \times session effect $F_{7,140} = 0.47$, $p > 0.05$), and exhibited lower biased responding (genotype effect $F_{1,20} = 12.21$, $p = 0.002$; genotype \times session interaction effect $F_{7,140} = 0.81$, $p > 0.05$), while the number of ITI responses made (genotype effect $F_{1,20} = 0.12$; genotype \times session effect $F_{7,140} = 0.81$, both p 's > 0.050) and incorrect response latencies (genotype effect $F_{1,20} = 2.84$; genotype \times session effect $F_{7,140} = 0.86$, both p 's > 0.050) failed to show a difference. Errors of omission showed a tendency for an increase in transgenic animals only (genotype effect $F_{1,20} = 3.93$, $p = 0.061$; genotype \times session effect $F_{7,140} = 0.91$, $p > 0.05$) (Table 8.1).

Subsequently, animals received nine more sessions under basal conditions, i.e., without tone presentation. Again, CRH overexpressing mice displayed an accuracy deficit (genotype effect $F_{1,20} = 4.57$, $p = 0.045$; genotype \times session effect $F_{8,160} = 1.00$, $p > 0.05$), earned less pellets (genotype effect $F_{1,20} = 20.62$, $p < 0.001$; genotype \times session effect $F_{8,160} = 2.84$, $p = 0.043$) and made more errors of omission (genotype effect $F_{1,20} = 11.27$, $p = 0.003$; genotype \times session effect $F_{8,160} = 1.19$, $p > 0.05$). Analysis of biased responding revealed a tendency for a group difference (genotype effect $F_{1,20} = 3.47$, $p = 0.077$; genotype \times session effect $F_{8,160} = 1.03$, $p > 0.05$). Genotypes did not differ from each other in terms of the number of ITI responses

(genotype effect $F_{1,20} = 0.17$; genotype \times session effect $F_{8,160} = 1.21$, both p 's > 0.050). Furthermore, there was no more effect on response latencies (correct response latency, genotype effect $F_{1,20} = 2.43$; genotype \times session effect $F_{8,160} = 1.37$; incorrect response latency, genotype effect $F_{1,20} = 0.08$; genotype \times session effect $F_{8,160} = 0.58$, all p 's > 0.050) (Table 8.1).

Effects of mild stress

Comparison of the last session under baseline conditions with the first session where animals received an i.p. saline injection prior to testing revealed that stress exposure induced a small reduction in the number of reinforcers earned (session effect $F_{1,20} = 32.64$; $p < 0.001$) and increased the number of errors of omission (session effect $F_{1,20} = 18.44$; $p < 0.001$), while percentage correct responding even increased (session effect $F_{1,20} = 7.01$; $p = 0.015$) (Figures 8.4A – C). Furthermore, there was a tendency for incorrect response latencies to decrease as a result of stress exposure (session effect $F_{1,20} = 4.27$; $p = 0.052$). Other parameters remained unaffected by this manipulation (all p 's > 0.100).

Although transgenic mice continued to differ from controls in terms of reinforcers earned (genotype effect $F_{1,20} = 22.15$; $p < 0.001$) and percentage correct responding (genotype effect $F_{1,20} = 7.92$; $p = 0.011$) (Figures 8.4A, B), there were no interactions between genotype and stress (reinforcers earned, genotype \times session effect $F_{1,20} = 2.25$; percentage correct responses, genotype \times session effect $F_{1,20} = 0.39$; both p 's > 0.050). Groups did not differ in errors of omission (genotype effect $F_{1,20} = 2.81$; genotype \times session effect $F_{1,20} = 1.08$, both p 's > 0.050), ITI responses (genotype effect $F_{1,20} = 0.74$; genotype \times session effect $F_{1,20} = 0.31$, both p 's > 0.050), response

latencies (correct, genotype effect $F_{1,20} = 1.32$; genotype \times session effect $F_{1,20} = 0.54$; incorrect, genotype effect $F_{1,20} = 0.07$; genotype \times session effect $F_{1,20} = 0.12$, all p 's > 0.050), or response bias (genotype effect $F_{1,20} = 0.03$; genotype \times session effect $F_{1,20} = 0.18$, both p 's > 0.050) (Figures 8.4D – G).

Effects of acute diazepam treatment

Overall, transgenic mice continued to earn less reinforcers (genotype effect: $F_{1,19} = 98.69$, $p < 0.001$), to perform less accurate (genotype effect: $F_{1,19} = 11.42$, $p = 0.003$), made more errors of omission (genotype effect: $F_{1,19} = 32.24$, $p < 0.001$), made more ITI responses (genotype effect: $F_{1,19} = 12.04$, $p = 0.003$), and had stronger side bias (genotype effect: $F_{1,19} = 7.63$, $p = 0.012$). Although groups still failed to differ in correct response latency (genotype effect: $F_{1,19} = 2.95$, $p > 0.05$), transgenic mice now had significantly shorter incorrect response latencies (genotype effect: $F_{1,19} = 2.22$, $p = 0.003$).

Diazepam caused a dose-dependent increase in the number of reinforcers earned (treatment effect $F_{7,133} = 5.08$, $p = 0.006$), but no genotype \times treatment interaction effect was found for this measure (genotype \times treatment effect $F_{7,133} = 1.80$, $p > 0.05$) (Figure 8.5B). Although a significant genotype \times treatment interaction effect was seen in errors of omission (genotype \times treatment effect $F_{7,133} = 4.05$, $p < 0.014$), this effect failed to reach significance in the post hoc analysis, and an overall effect of treatment on omissions also just to reach failed significance (treatment effect $F_{7,133} = 2.75$, $p = 0.0505$) (Figure 8.5C). The percentage correct response remained unaffected by diazepam (treatment effect $F_{7,133} = 1.65$, $p > 0.05$) and no genotype \times treatment inter-

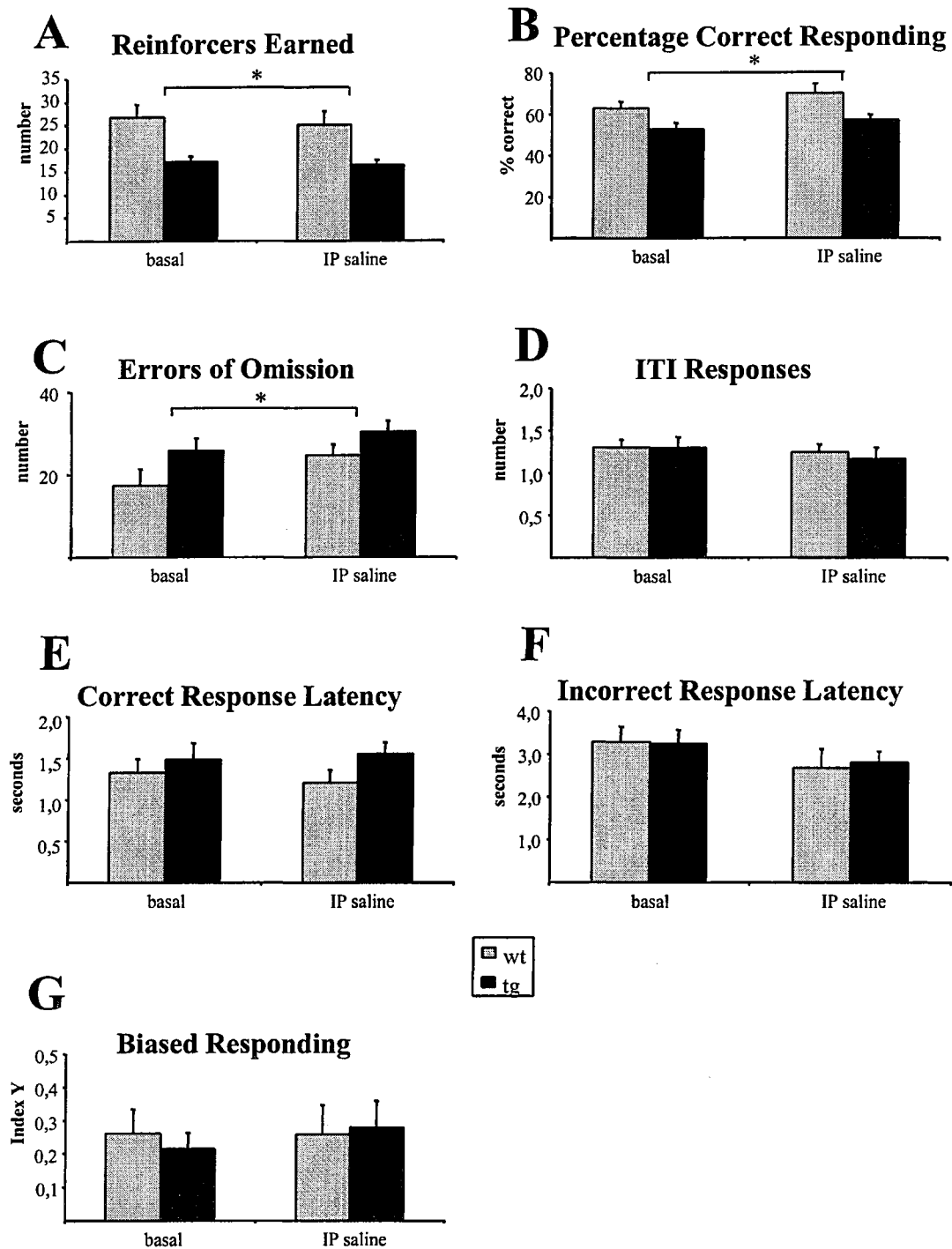


Figure 8.4 Effects of injection stress on 5-CSRTT performance in mice overproducing CRH. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding (index Y). Data are presented as means, with error bars denoting SEMs. * $p < 0.050$.

action was observed (genotype x treatment effect $F_{7,133} = 1.57$, $p > 0.05$) (Figure 8.5A). Treatment also failed to affect ITI responses (treatment effect $F_{7,133} = 1.12$, $p > 0.05$). However, an interaction between genotype and treatment with diazepam was found (genotype x treatment effect $F_{7,133} = 6.78$, $p < 0.001$) (Figure 8.5D). Post hoc analysis indicated that the two groups failed to differ at low doses up to 1.0 mg/kg, but that transgenic mice showed significantly increased ITI responding at higher doses, which may be indicative for behavioural disinhibition. No effects of treatment were seen on latencies or biased responding (correct latency: treatment effect $F_{7,133} = 1.11$; genotype x treatment effect $F_{7,133} = 1.73$; incorrect latency: treatment effect $F_{7,133} = 1.81$, genotype x treatment effect $F_{7,133} = 0.50$; biased responding: treatment effect $F_{7,133} = 1.26$; genotype x treatment effect $F_{7,133} = 1.51$, all p 's > 0.050).

Effects of acute R121919 treatment

Transgenic mice continued to perform below wildtype level, i.e., earned less reinforcers (genotype effect $F_{1,15} = 12.51$, $p = 0.003$) and percentage correct responses were lower (genotype effect $F_{1,15} = 25.78$, $p < 0.001$). There was a strong tendency for transgenics to make more omission (genotype effect: $F_{1,15} = 1.78$, $p = 0.0501$), while ITI responses (genotype effect $F_{1,15} = 0.14$, $p > 0.05$) and side bias (genotype effect $F_{1,15} = 0.49$, $p > 0.05$) again failed to differ between groups. However genotypes continued to differ in response latencies (correct response latencies: genotype effect $F_{1,15} = 25.78$, $p = 0.021$; incorrect response latencies: genotype effect $F_{1,15} = 6.90$, $p = 0.020$).

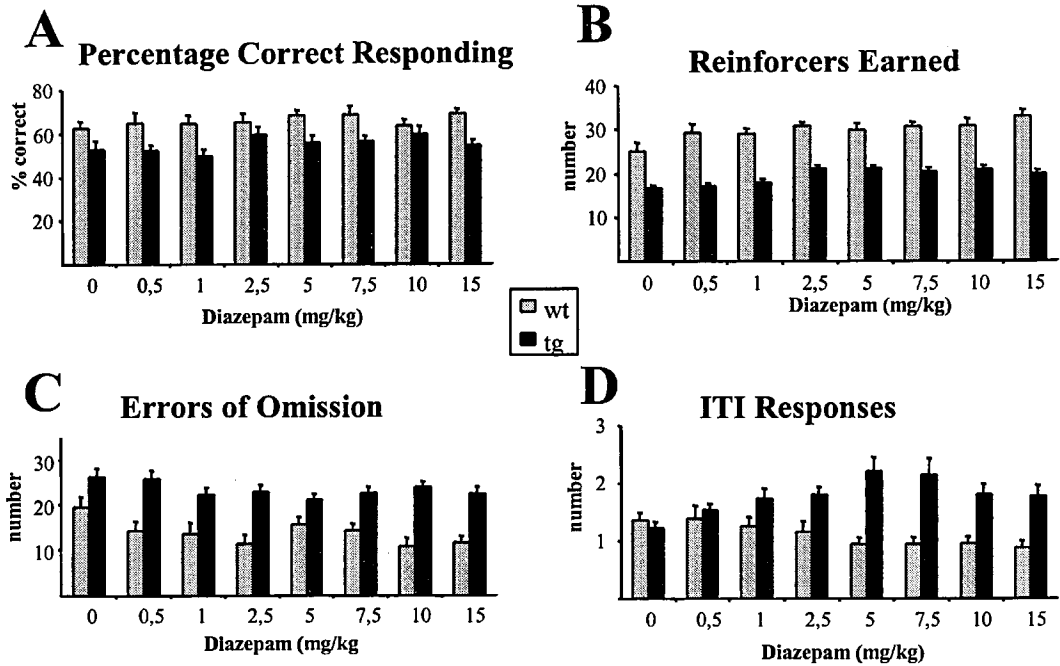


Figure 8.5 Effects of diazepam on 5-CSRTT performance in mice overproducing CRH. Diazepam was administered at dosages of 0.0, 0.5, 1.0, 2.5, 5.0, 7.5, 10.0 and 15.0 mg/kg i.p., 20 min prior to testing. Treatment given in ascending order. (A) Percentage correct responding, (B) number of reinforcers earned, (C) number of errors of omission, (D) ITI responses per trial. Data are presented as means, with error bars denoting SEMs.

Blockade of CRHR1 by R121919 gradually reduced the number of reinforcers earned (treatment effect $F_{3,45} = 5.28$, $p < 0.013$), but no interaction between genotype and treatment was observed (genotype x treatment effect $F_{3,45} = 2.41$ $p > 0.05$) (Figure 8.6B). At the same time, CRHR1 blockade increased the number of errors of omission made (treatment effect $F_{3,45} = 6.35$, $p = 0.007$) but, again, no genotype x treatment interaction effect was found (genotype x treatment effect $F_{3,45} = 1.76$, $p > 0.05$) (Figure 8.6C). R121919 had no effects on percentage correct responding (treatment effect $F_{3,45} = 0.34$; genotype x treatment effect $F_{3,45} = 0.44$, both p 's > 0.050) (Figure 8.6C). Likewise, treatment with R121919 failed to affect ITI responses (treatment effect $F_{3,45} = 1.53$; genotype x treatment, $F_{3,45} = 1.57$, both p 's $>$

0.050), correct (treatment effect $F_{3,45} = 1.32$; genotype x treatment, $F_{3,45} = 3.03$, both p 's > 0.050) and incorrect response latencies (treatment effect $F_{3,45} = 0.97$; genotype x treatment, $F_{3,45} = 3.64$, both p 's > 0.050), and biased responding (treatment effect $F_{3,45} = 1.75$; genotype x treatment effect $F_{3,45} = 0.62$, both p 's > 0.050).

Effects of acute scopolamine treatment

Percentage correct responses (genotype effect $F_{1,13} = 5.33$, $p = 0.038$) and the number of reinforcers earned (genotype effect $F_{1,13} = 18.32$, $p = 0.001$) continued to be lower in transgenic animals during this experimental stage. Transgenic animals again made significantly more errors of omission (genotype effect $F_{1,13} = 7.86$, $p = 0.016$), while no difference was seen in any of the other measures (ITI responses $F_{1,13} = 0.62$; side bias $F_{1,13} = 4.50$, correct response latencies $F_{1,13} = 1.79$; incorrect response latencies $F_{1,13} = 0.57$; all p 's > 0.050).

There was an overall effect of treatment on percentage correct responding, with scopolamine leading to reduced accuracy (treatment effect $F_{3,39} = 4.90$, $p = 0.014$), and this effect was comparable between the two groups (genotype x treatment effect $F_{3,39} = 0.40$, $p > 0.05$) (Figure 8.7A). Likewise, scopolamine reduced the number of reinforcers earned (treatment effect $F_{3,39} = 60.76$, $p < 0.001$). In addition, an interaction between genotype and treatment was seen (treatment $F_{3,39} = 3.76$, $p = 0.040$). Although post hoc testing revealed significant group differences at every dose of scopolamine tested, further inspection of the data indicated that wildtype mice showed a steeper dose-dependent decline in reinforcers earned than transgenic

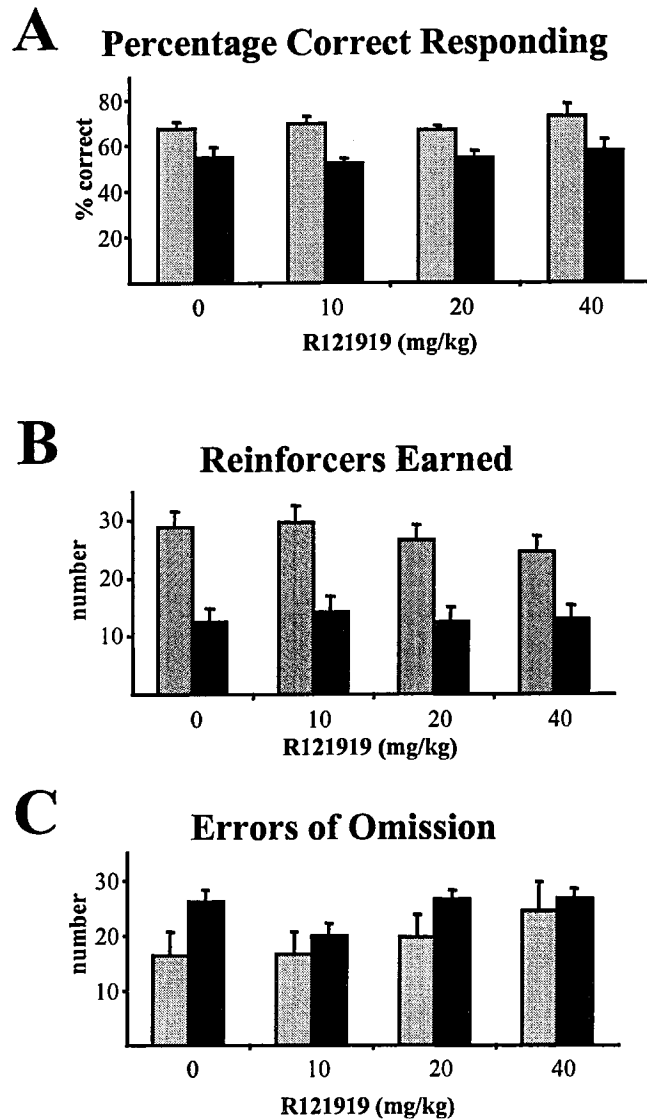


Figure 8.6 Effects of R121919 on 5-CSRTT performance in mice overproducing CRH. R121919 was administered at dosages of 0.0, 10.0, 20.0 and 40.0 mg/kg i.p., 20 min prior to testing. Treatment given in ascending order. (A) Percentage correct responding, (B) number of reinforcers earned, (C) number of errors of omission. Data are presented as means, with error bars denoting SEMs.

animals (Figure 8.7B). Scopolamine also increased errors of omission, but this effect was again similar in both groups (treatment effect $F_{3,39} = 57.59$, $p < 0.001$; genotype x treatment effect $F_{3,39} = 1.25$, $p > 0.05$) (Figure 8.7C). ITI responses were decreased by treatment (treatment effect $F_{3,39} = 20.88$, $p < 0.001$), but no genotype x treatment interaction was observed (genotype x treatment effect $F_{3,39} = 1.14$, $p > 0.05$). Overall, there was no effect of treatment on correct response latencies (treatment effect $F_{3,39} = 1.68$, $p > 0.05$). A genotype x treatment interaction was observed for this measure (genotype x treatment effect $F_{3,39} = 3.53$, $p = 0.049$), but post hoc analysis again failed to reveal further differences (Figure 8.7D). Treatment with scopolamine had no effect on incorrect response latencies (treatment effect $F_{3,39} = 1.57$; genotype x treatment effect $F_{3,39} = 0.72$, both p 's > 0.050) or biased responding (treatment effect $F_{3,39} = 2.88$; genotype x treatment effect $F_{3,39} = 1.79$, both p 's > 0.050).

Effects of acute physostigmine treatment

Overall effects of genotype were comparable to the previous stage of testing: percentage correct responses continued to be lower in transgenics (genotype effect $F_{1,15} = 9.03$, $p = 0.009$), whereas the number of reinforcers earned was decreased in the mutants (genotype effect $F_{1,16} = 6.88$, $p = 0.018$). No significant differences between groups were found in terms of ITI responses (genotype effect $F_{1,16} = 2.00$), side bias (genotype effect $F_{1,14} = 0.14$), correct response latencies: ($F_{1,16} = 3.97$), and incorrect response latencies (genotype effect $F_{1,16} = 0.04$) (all p 's > 0.050). Only errors of omission again failed to significantly differ between groups (genotype effect $F_{1,16} = 0.22$, $p > 0.05$).

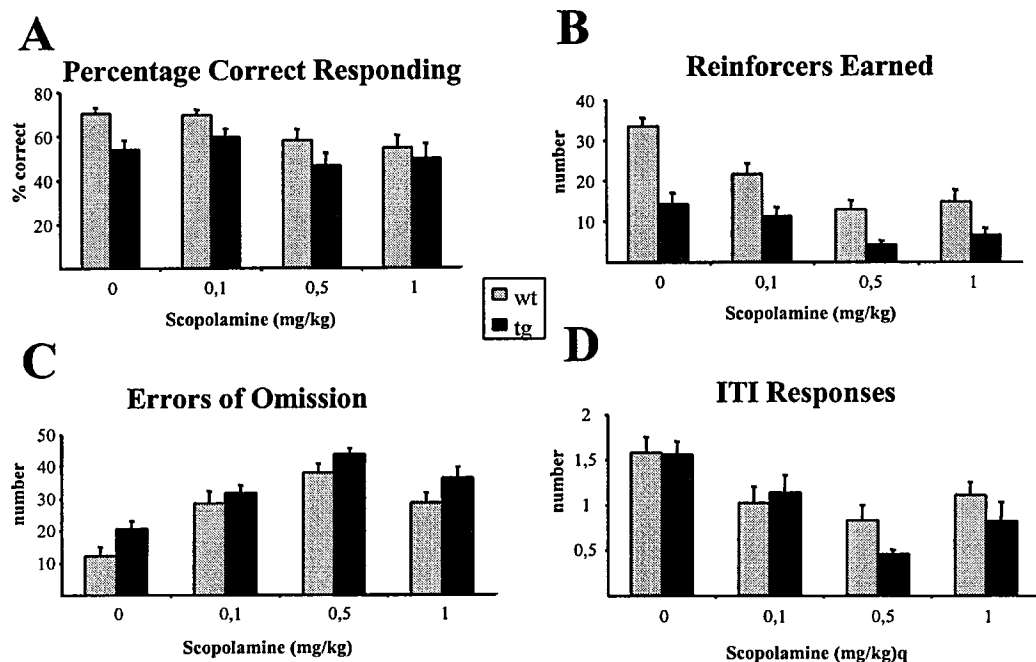


Figure 8.7 Effects of scopolamine on 5-CSRTT performance in mice overproducing CRH. Scopolamine was administered at dosages of 0.0, 0.1, 0.5 and 1.0 mg/kg i.p., 20 min prior to testing. Treatment given in ascending order. (A) percentage correct responding, (B) number of reinforcers earned, (C) number of errors of omission, (D) ITI responses per trial. Data are presented as means, with error bars denoting SEMs:

At high physostigmine doses (0.5 mg/kg), wildtype animals earned less reinforcers than transgenic mice, suggesting again decreased sensitivity of CRH overexpressors to cholinergic manipulation, i.e., to the response depressing effects of the drug (Figure 8.8A). This suggestion was confirmed by the errors of omission measure: the number of errors of omission was affected by treatment (treatment effect $F_{4,64} = 79.13$, $p > 0.001$), and there was a significant genotype x treatment interaction effect (genotype x treatment effect $F_{4,64} = 14.07$, $p > 0.001$). Post hoc testing revealed that wildtype animals made less errors of omission after saline and 0.05 mg/kg physostigmine administration, while groups did not differ after treatment with intermediate doses (0.1 and 0.25 mg/kg) of physostigmine. However, wildtype

animals made more errors of omission after the high dose (0.5 mg/kg) administration (Figure 8.8C). Consequently, the number of reinforcers earned was decreased by treatment (treatment effect $F_{4,64} = 93.03$, $p < 0.001$). A genotype x treatment interaction effect was observed (genotype x treatment effect $F_{4,64} = 22.93$, $p < 0.001$), and post hoc testing revealed that wildtype animals earned more reinforcers after saline and low dose (0.05 mg/kg) physostigmine administration than transgenic mice, while groups did not differ at intermediate doses (0.1 and 0.25 mg/kg) of physostigmine (Figure 8.8A). Furthermore, ITI responses were dose-dependently decreased after AChE blockade (treatment effect $F_{4,64} = 24.50$, $p < 0.001$), while no genotype x treatment interaction effect was found (genotype x treatment effect $F_{4,64} = 1.10$, $p > 0.05$) (Figure 8.8D). Because of the high number of omissions made under treatment with 0.5 mg/kg physostigmine, data obtained under this treatment were excluded from analysis of percentage correct responses and latencies. The highest dose of physostigmine analysed (0.25 mg/kg) left percentage correct responding unaffected (treatment effect $F_{3,45} = 0.12$; genotype x treatment effect $F_{3,45} = 0.42$, both p 's > 0.050) (Figure 8.8B). Both correct (treatment effect $F_{3,48} = 1.46$; genotype x treatment effect $F_{3,48} = 2.16$, both p 's > 0.050) and incorrect choice latencies (treatment effect $F_{3,48} = 0.16$, $p > 0.05$; genotype x treatment effect $F_{3,48} = 1.64$, both p 's > 0.050) also remained unaffected by treatment with physostigmine. Finally, biased responding increased as a function of treatment (treatment effect $F_{3,42} = 4.56$, $p < 0.024$), and again no genotype x treatment interaction effect was seen (genotype x treatment effect $F_{3,42} = 1.80$, $p > 0.05$).

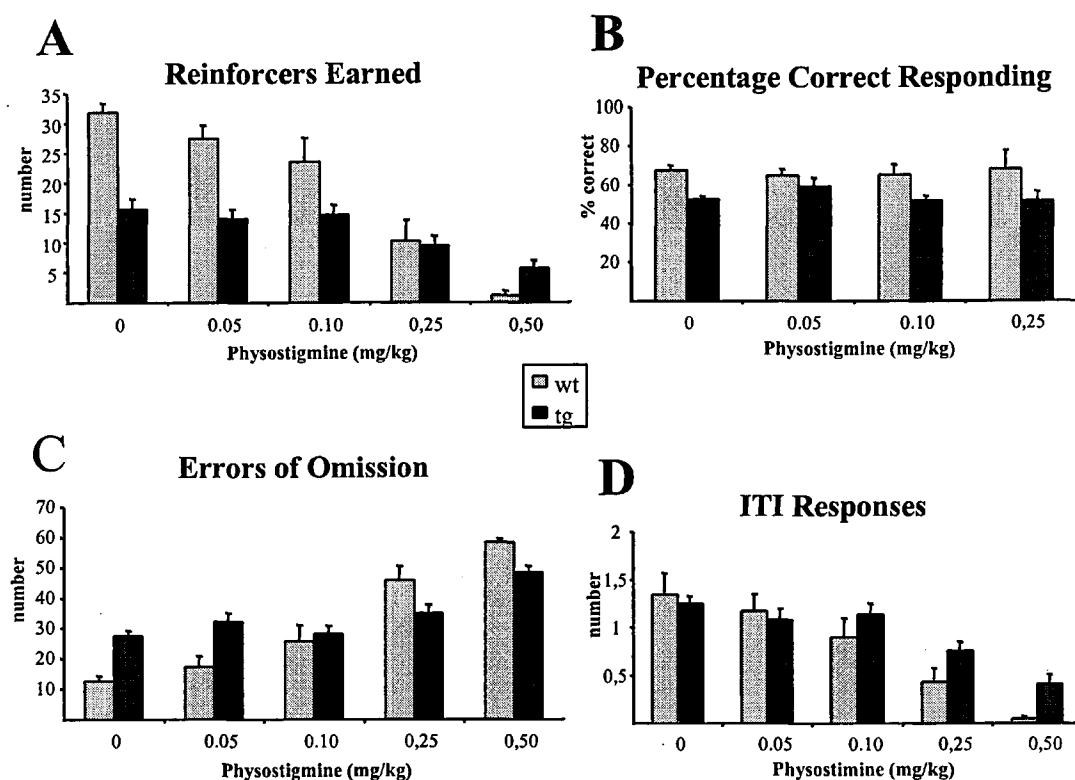


Figure 8.8 Effects of physostigmine on 5-CSRTT performance in mice overproducing CRH. Physostigmine was administered at dosages of 0.0, 0.05, 0.10, 0.25 and 0.50 mg/kg i.p., 20 min prior to testing. Treatment given in ascending order. Data is shown for (A) percentage correct responding, (B) number of reinforcers earned, (C) number of errors of omission, (D) ITI responses per trial. Data are presented as means, with error bars denoting SEMs.

Discussion

CRH overexpressing mice showed impaired autoshaping of a nose-poke response to a light stimulus, as indicated by retarded, but not absent, association between the stimulus and reward and reduced responsivity. Although subsequent simultaneous discrimination training indicated that transgenic animals had learned some association as they performed above chance level from the first session onwards, they clearly performed below wildtype level. However, both groups of animals improved performance over session, indicating the ability to learn the discrimination in transgenic animals, although they continued to respond with lower accuracy. At

least part of this may be due to different baselines carried over from autoshaping. In addition, biased responding was increased during acquisition and subsequent experimental stages, indicating that transgenic animals reverted to a more simple response strategy.

Extended training with long stimulus durations revealed that transgenic animals were able to reach similar accuracy levels as wildtype animals, although responsivity continued to be reduced, as indicated by a reduction in the number of reinforcers earned, increased errors of omission, decreased responsivity during the ITI, and increased response latencies. Once transgenic mice had reached an accuracy level that was comparable to wildtype performance, they were also able to maintain accuracy to similar degree as wildtypes over a wide range of different stimulus durations. This indicates that learning the discrimination was retarded but remembrance of the response rule appeared to be intact once the discrimination was acquired. Increasing attentional load by decreasing the stimulus duration led to a decrease in percentage correct responses and increased biased responding in both groups.

When animals were tested for an extended period of 17 sessions with shortest stimulus duration (0.5 s), a mild but significant impairment in percentage correct responding re-emerged in transgenic animals. This was accompanied by longer correct response latencies, while incorrect latencies (which were longer than correct response latencies in both genotypes) did not differ between groups. This accuracy deficit was independent on whether or not a pre-sound was present, suggesting that

CRH overexpressing mice did not benefit from this manipulation guiding stimulus detection (cued detection, Bushnell, 1995).

Exposure to a mild stressor (i.p. injection of saline) impaired performance on 5-CSRTT, as indicated by a reduction in the number of reinforcers earned and an increase in errors of omission. Interestingly, this resulted in an overall increase in percentage correct responding. In other words, animals showed a stronger tendency to refrain from responding, which consequently led to a net decrease in reinforcers earned but, at the same time, animals responded more accurately once responses occurred. However, this effect was mild, questioning the behavioural significance of this effect.

The effects of diazepam were small, leaving accuracy unaffected and, if at all, causing release of responding, as evidenced by an increase in the number of reinforcers earned and an effect on errors of omission which just failed significance.

Administration of the CRHR1 antagonist R121919 reduced responsivity, as indicated by increased omissions and decreased reinforcers earned. However, R121919 failed to improve performance in transgenic animals, suggesting that the deficit seen in the overexpressors was not mediated through overactivation of this receptor subtype.

Manipulation of the cholinergic system, however, affected both transgenic and wildtype animals to different degree. Although the effects of scopolamine on accuracy were comparable between groups, the effects of scopolamine on the number of reinforcers earned were more pronounced in wildtype mice. Likewise,

physostigmine had stronger effects in wildtype than in transgenic animals at high doses (0.5 mg/kg), suggesting again decreased sensitivity of CRH overexpressors to cholinergic manipulation, i.e., to the response depressing effects of the drug. This suggestion was confirmed by the errors of omission and the number of reinforcers measures. Since transgenic mice were hyporeactive to both blockade and indirect stimulation of cholinergic receptors, the possibility of downregulation of cholinergic receptors due to CRH-induced increases in cholinergic activity (Day et al., 1998a,b) must be considered, although direct evidence is missing.

Chapter 9

Effects of lack of CRH on 5-choice serial reaction time task performance

Introduction

Overproduction of CRH resulted in mild disruption of visuospatial attentional processes, assessed in the 5-CSRRT (Chapter 8). Therefore, lack of CRH might result in an improvement in these processes. An alternative possibility, however, could be that overproduction of CRH might induce overarousal, whereas a lack of CRH might result in an insufficient arousal state, again impairing attentional processes. In this chapter, the necessity of CRH in attentional processes is addressed by investigating the performance of mice lacking CRH in the 5-CSRTT. Furthermore, the effects of lack of CRH may be more distinct after a stressful situation, because such situations activate the CRH system. Therefore, 5-CSRTT performance was also assessed after two distinct stressors: social defeat and inescapable swim stress.

Material and methods

Animals

Breeding stock for wildtype mice and knockout male mice for the null CRH allele were derived from heterozygote breeding pairs (purchased from the Jackson Laboratory (mixed background 129SV/J C57BL/6), originally generated by Muglia and colleagues (1995), bred and reared in the animal house of the Max Planck Institute. Animals, both groups 7 males, were housed individually and maintained on a 12:12 h light/dark cycle (lights on at 06:00 hours) with *ad lib* water throughout. They were maintained at 85-90% of their free feeding weight, starting one week prior to the beginning of the experiment by restricting the laboratory chow access to 3.0-3.5 g / day / mouse (depending on the number of pellets eaten during testing). All experiments were conducted during the light phase of the cycle.

Behavioural procedure

5-CSRRT

Testing took place during the light phase of the light/dark cycle. Animals were trained to stable baseline performance as described in Chapters 2 and 8.

Manipulations of basic task parameters

After animals reached stable performance, a series of manipulations of the basic task were made which were designed to increase the attentional load. First, the ITI durations were gradually reduced (5.0, 2.5, 1.0 seconds) after which they were

increased (5.0, 7.5, 10 seconds). Second, the effects of the presence of a distractor (a white noise stimulus (1.2 kHz, 76 dB), presented during the 500 ms preceding the stimulus onset, were studied.

Effects of social defeat on 5-CSRTT performance

Animals were placed in the home cage of an older single caged mouse (resident) for 15 min. The mice were separated by a metal wire fence, directly after the first aggressive interaction. After the period of 15 min, animals were returned to their home cage for an additional 20 min, after which they were tested in the 5-CSRTT.

Effects of inescapable swim stress on 5-CSRTT performance

Animals were exposed to inescapable swim stress for 6 min (see Chapter 2), after which they were returned to their home cage for an additional 20 min, followed by a session in the 5-CSRTT.

Effects of acute scopolamine treatment

Finally, animals were treated with the muscarinic antagonist scopolamine (0.0, 0.1, 0.5, 1.0, 2.0 mg/kg i.p., 20 min prior to test; treatment given in ascending order, with saline as vehicle). At least two days lapsed between drug testing in order to allow for sufficient wash-out and recovery of the animals.

Results

Autoshaping procedure

The number of trials completed did differ between groups ($F_{1,12} = 1.54$, $p > 0.05$). No effect of session was found (session effect $F_{6,72} = 0.50$; genotype x session effect $F_{6,72} = 1.00$, all p 's > 0.050) (Figure 9.1A). Likewise, no difference in ITI responses per trial were found (genotype effect $F_{1,12} = 0.49$; session effect $F_{6,66} = 1.55$; genotype x session effect $F_{6,66} = 1.64$, all p 's > 0.050) (Figure 9.1B). Furthermore, no genotype, session effect, or interaction of both was found in terms of the time to complete a trial, (genotype effect $F_{1,10} = 0.49$; session effect $F_{6,60} = 1.82$; genotype x session effect $F_{6,60} = 1.57$, all p 's > 0.050) and trials completed prior to pellet delivery (genotype effect $F_{1,12} = 0.49$; session effect $F_{6,66} = 0.30$; genotype x session effect $F_{6,66} = 1.04$, all p 's > 0.050) (Figures 9.1C, D).

Simple visual discrimination learning

During the initial stage of visual discrimination, the stimulus duration was 8 sec. Both groups of animals responded above chance from the first session onwards, indicating that they had already learned to associate the light stimulus with reinforcement during autoshaping. Although both groups reached at least at 70 % correct, respondings during the last two sessions, the session effect just failed to reach significance (session effect $F_{6,72} = 3.77$, $p = 0.054$) (Figure 9.2B). No significant group effect ($F_{1,12} = 2.52$), or genotype \times session interaction was observed ($F_{(6,72)} = 2.14$, both p 's > 0.050) for this parameter. Likewise no significant differences were found in terms of errors of omission (genotype effect $F_{1,12} = 2.48$; session \times genotype effect $F_{6,72} = 1.32$), reinforcers earned (genotype effect $F_{1,12} =$

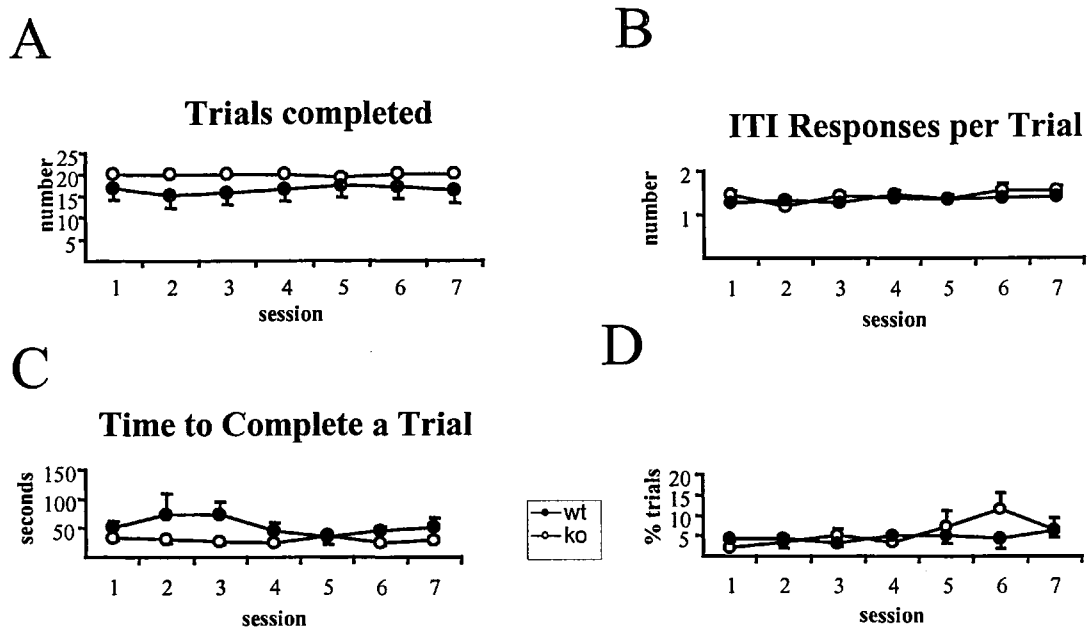


Figure 9.1 Effects of lack of CRH on performance during autoshaping. Animal were tested for 7 successive sessions. (wt) wildtype mice, (ko) CHR knockout mice. (A) number of trials completed, (B) ITI responses per trial, (C) time to complete a trial, (D) trials completed prior to pellet delivery. Data are presented as means, with error bars denoting SEMs.

2.96; session \times genotype effect $F_{6,72} = 2.42$), ITI responses (genotype effect $F_{1,12} = 0.61$; session \times genotype effect $F_{6,72} = 21.45$), correct response latency (genotype effect $F_{1,12} = 0.70$; session \times genotype effect, $F_{6,72} = 1.69$) and incorrect response latency (genotype effect, $F_{1,12} = 4.69$; session \times genotype effect, $F_{6,72} = 0.88$) (all p 's > 0.05 , Figures 9.2A, C, D, E, F). In addition, although no overall groups difference was found in biased responding (genotype effect $F_{1,12} = 1.11$, $p > 0.05$), a session \times genotype interaction was found (effect $F_{6,72} = 3.41$, $p = 0.023$). Post hoc testing revealed that CRH ko mice showed reduced biased responding, during sessions 5 and 6 (both p 's < 0.050).

Reduction of stimulus duration

Next, the stimulus was decreased (4, 2, 1 and 0.5 sec, 5 trials per stimulus duration). Overall, percentage correct responses ($F_{3,33} = 88.22$, $p < 0.001$) and number of reinforcers earned (stimulus duration effect $F_{3,33} = 9.48$, $p < 0.001$) decreased in a manner dependent on stimulus duration (Figures 9.3A, B). Despite the increasing attentional load, errors of omission also declined with decreasing stimulus duration (stimulus duration effect $F_{3,33} = 12.99$, $p < 0.001$) (Figure 9.3C). The number of responses made during the ITI remained unaffected by stimulus duration ($F_{3,33} = 2.04$, $p > 0.05$), while both correct and incorrect latencies decreased with decreasing stimulus ($F_{3,33} = 35.79$, $p < 0.001$ and $F_{3,33} = 7.22$, $p < 0.006$, respectively) (Figures 9.3D, E, F). Moreover, biased responding increased as a consequence of decreasing stimulus duration ($F_{3,33} = 8.61$, $p < 0.003$) (Figure 9.3G).

No genotype-dependent differences were found during this experimental: percentage correct responding (genotype effect $F_{1,11} = 0.62$; genotype \times stimulus duration effect, $F_{3,33} = 1.59$; genotype \times stimulus duration \times session effect $F_{12,132} = 1.27$), reinforcers earned (genotype effect $F_{1,11} = 0.09$; genotype \times stimulus duration effect $F_{3,33} = 0.29$; genotype \times stimulus duration \times session effect $F_{12,132} = 1.48$), errors of omission made (genotype effect $F_{1,11} = 0.00$; genotype \times stimulus duration effect $F_{3,33} = 0.55$; genotype \times stimulus duration \times session effect $F_{12,132} = 1.34$), number of ITI responses

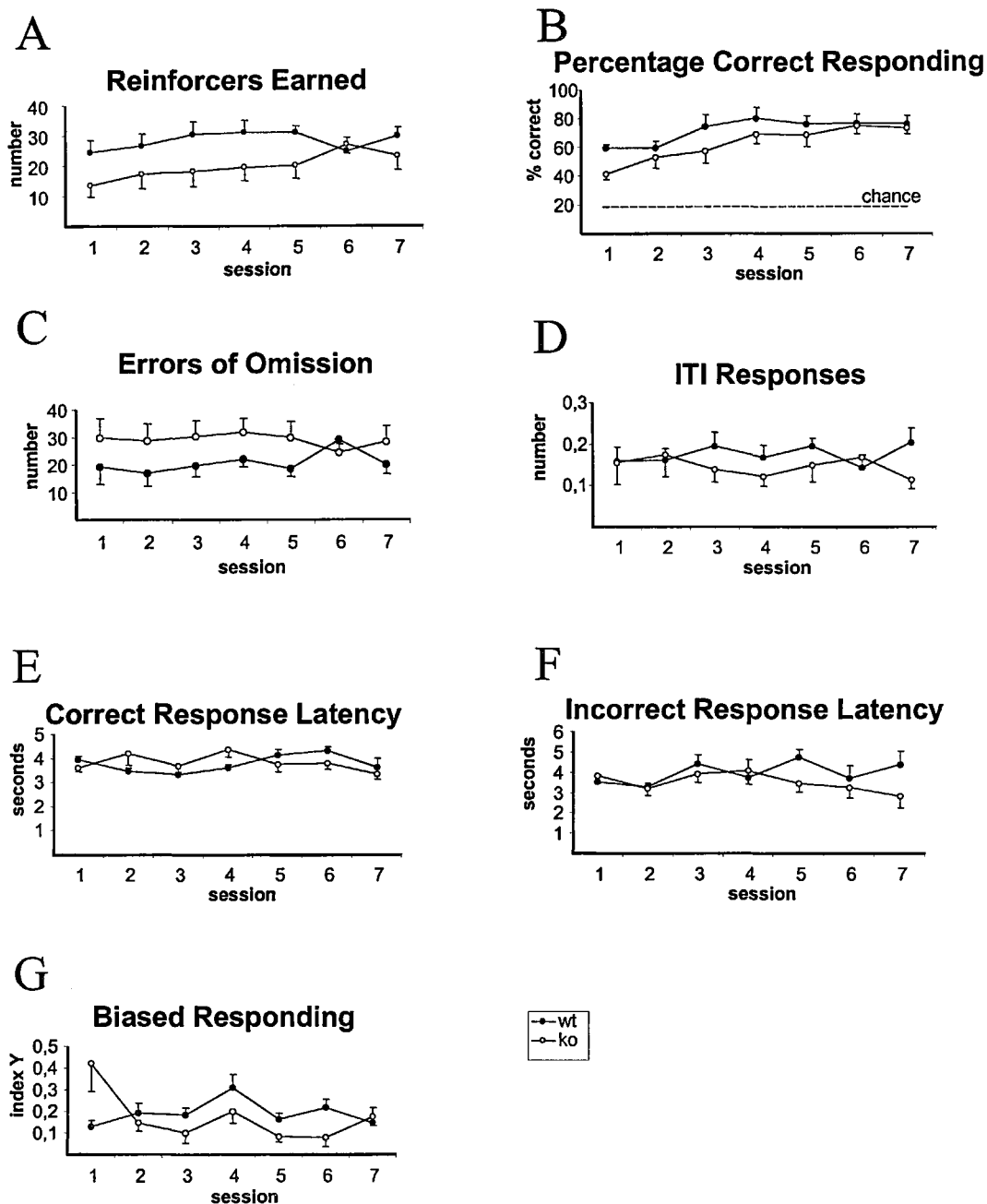


Figure 9.2 Five-choice simultaneous discrimination in mice lacking CRH. Animals were tested for 7 successive sessions, with a stimulus duration of 8 sec. (wt) wildtype mice, (ko) CHR knockout mice. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

(genotype effect $F_{1,11} = 1.71$; genotype \times stimulus duration effect $F_{3,33} = 0.40$; genotype \times stimulus duration \times session effect $F_{12,132} = 0.89$), correct response latency (genotype effect $F_{1,11} = 2.45$; genotype \times stimulus duration effect $F_{3,33} = 1.03$; genotype \times stimulus duration \times session effect $F_{12,132} = 1.36$), incorrect response latencies (genotype effect $F_{1,11} = 0.05$; genotype \times stimulus duration effect $F_{3,33} = 0.14$; genotype \times stimulus duration \times session effect $F_{12,132} = 0.89$) and biased responding (genotype effect $F_{1,11} = 0.05$; genotype \times stimulus duration effect $F_{3,33} = 0.48$; genotype \times stimulus duration \times session effect $F_{12,132} = 0.55$) (all p 's > 0.05 ; Figures 9.3A-G).

Baseline performance

Subsequently, mice received fifteen more sessions with 0.5 s stimulus duration. Over repeated testing with this stimulus duration (twenty sessions, including the first 5 sessions), no significant differences were found in the performance of knockout mice compared to wildtype littermates (Table 9.1).

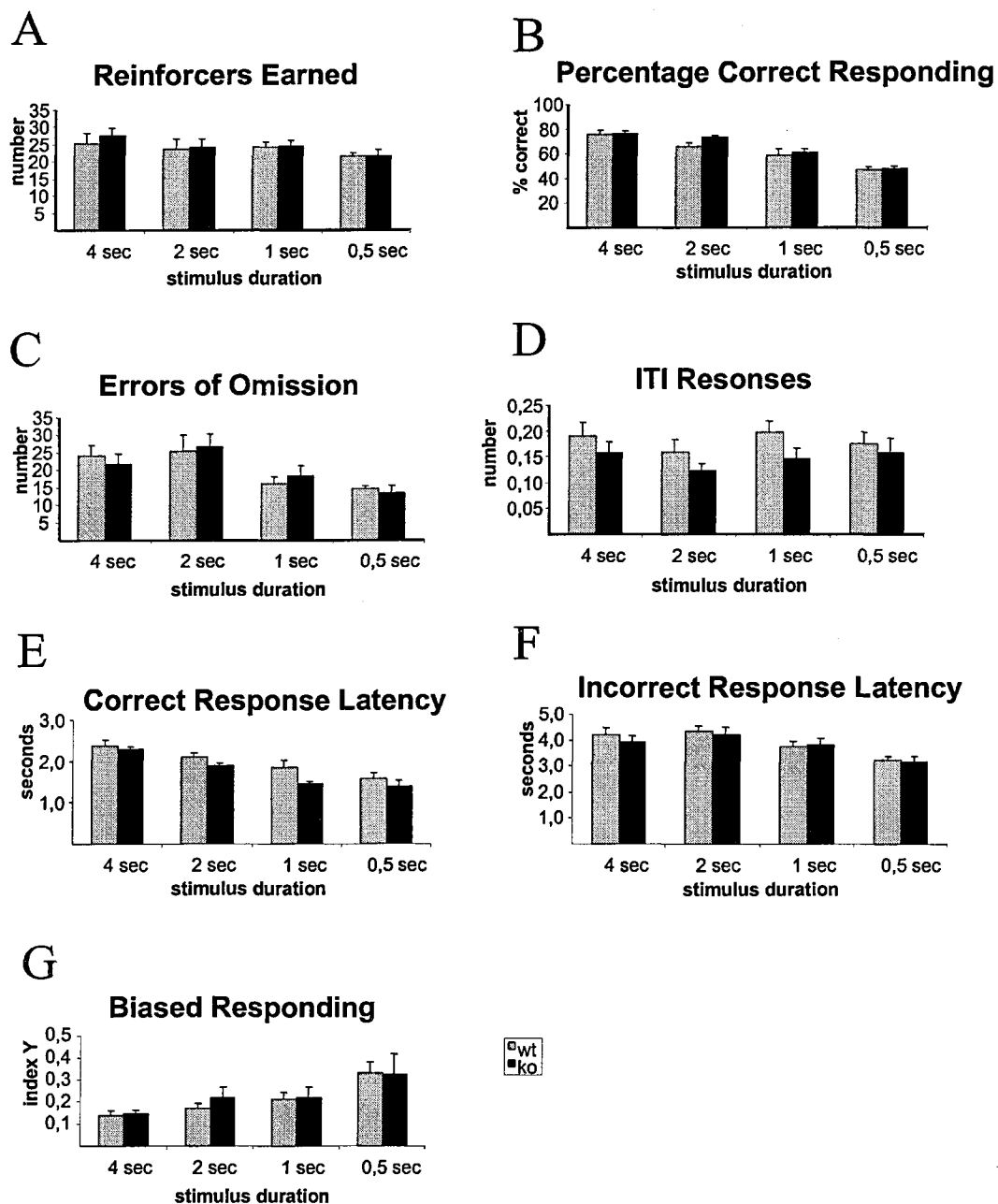


Figure 9.3 Effects of gradual reduction of the stimulus duration on 5-CSRTT performance in mice lacking CRH. Animals were tested for 5 sessions for each stimulus duration. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

Table 9.1 5-CSRRT performance in mice lacking CRH under basal conditions (data are expressed as means \pm SEMs).

measure	wildtype	knockout	genotype effect $F_{1,11}$	genotype \times session $F_{19,209}$
Reinforcers Earned	22.07 \pm 1.21	22.18 \pm 1.61	0.00	1.31
% Correct Responses	51.13 \pm 1.77	51.19 \pm 2.60	0.00	1.34
Errors of Omission	16.59 \pm 1.40	16.70 \pm 1.60	0.00	0.91
No. ITI Responses	0.15 \pm 0.02	0.13 \pm 0.02	0.51	0.59
Corr. Resp. Lat. (sec)	1.49 \pm 0.07	1.30 \pm 0.09	2.98	1.33
Incorr. Resp. Lat. (sec)	3.16 \pm 0.12	2.94 \pm 0.18	1.11	1.40
Bias (Index Y)	0.32 \pm 0.04	0.27 \pm 0.03	0.66	0.71

All p 's > 0.050 ; No.: number; Corr. Resp. Lat.: correct response latency; Incorr. Resp. Lat.: incorrect response latency.

White noise distractor

Presenting white noise before the start of a trial did not affect the overall number of reinforcers earned ($F_{1,11} = 0.73$, $p > 0.05$). The errors of omission, however, were increased by introduction of white noise ($F_{1,11} = 8.31$, $p = 0.015$), whereas the percentage correct respondings tended to be increased, but just failed significance ($F_{1,11} = 3.83$, $p = 0.076$) (Figures 9.4A, B). Correct response latency was not affected by the auditory disturbance ($F_{1,11} = 0.02$, $p > 0.05$), whilst incorrect response latency

tended to be increased (noise effect $F_{1,11} = 4.44$, $p = 0.059$) (Figures 9.4C, D). Presentation of white noise did not affect ITI responses ($F_{1,11} = 0.21$, $p > 0.05$) and biased responses ($F_{1,10} = 0.12$, $p > 0.05$).

No differences of genotype were found in this experimental phase: percentage correct responding (genotype effect $F_{1,11} = 0.21$; genotype x noise effect $F_{1,11} = 1.31$), reinforcers earned (genotype effect $F_{1,11} = 0.00$; genotype x noise effect $F_{1,11} = 0.12$), errors of omission made (genotype effect $F_{1,11} = 0.06$; genotype x noise effect $F_{1,11} = 0.40$), correct response latency (genotype effect $F_{1,11} = 0.02$; genotype x noise effect $F_{1,11} = 0.05$), incorrect response latency (genotype effect $F_{1,11} = 0.90$; genotype x noise effect $F_{1,11} = 0.15$), ITI responses (genotype effect $F_{1,11} = 1.49$; genotype x noise effect $F_{1,11} = 0.02$) and biased responses (genotype effect $F_{1,11} = 1.73$; genotype x noise effect $F_{1,11} = 0.13$) (all p 's > 0.050).

Alterations of ITI duration

Increasing the ITI duration reduced the number of reinforcers earned $F_{2,22} = 5.76$, $p < 0.050$) (Figure 9.5B). Post hoc analyses revealed no significant differences between individual ITI durations. Reducing the ITI duration had no effect on the reinforcers earned ($F_{2,22} = 2.22$, $p > 0.05$) (Figure 9.5A). Furthermore, altering the ITI did not affect other parameters: percentage correct responding (ITI reduction effect $F_{2,22} = 0.16$; ITI increment effect $F_{2,22} = 1.22$), errors of omission (ITI reduction effect $F_{2,22} = 2.10$; ITI increment effect $F_{2,22} = 3.81$), correct response latency (ITI reduction effect $F_{2,22} = 0.16$; ITI increment effect $F_{2,22} = 0.59$), incorrect response latency (ITI reduction effect $F_{2,22} = 0.79$; ITI increment effect $F_{2,22} = 0.55$), and biased responding (ITI reduction effect $F_{2,22} = 0.60$; ITI increment effect $F_{2,22} = 0.55$) (all p 's > 0.050).

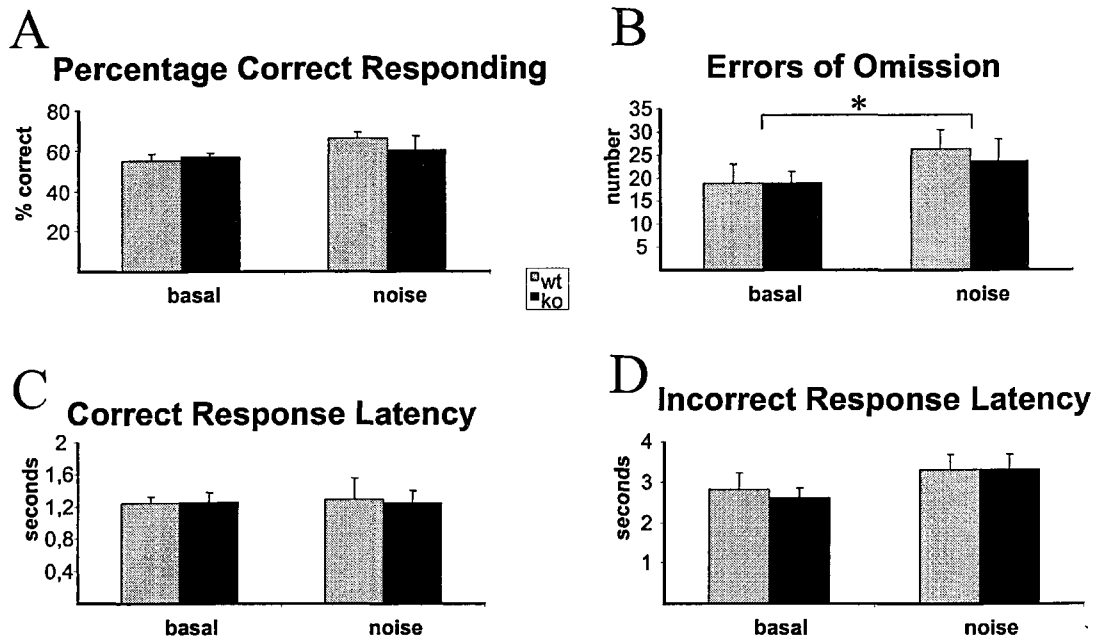


Figure 9.4 Effects of white noise distraction on 5-CSRTT performance in mice lacking CRH. (A) percentage correct responding, (B) number of errors of omission, (C) correct response latency, (D) incorrect response latency. Data are presented as means, with error bars denoting SEMs. * $p < 0.050$.

A significant genotype effect was found during the ITI duration reduction phase in terms of correct response latency ($F_{1,11} = 0.79$, $p < 0.050$). However, post hoc analyses did not reveal any significant differences between individual ITI durations (Figure 9.5C). Visual inspection of the data revealed that during this phase the most distinct difference between groups was found when animals were tested with an ITI of 5 seconds, suggesting that reducing the ITI is not the cause of the group difference found. No genotype x ITI reduction interaction was found ($F_{2,22} = 0.20$, $p > 0.05$) nor effects of increasing the ITI duration (genotype effect $F_{1,11} = 0.86$; genotype x ITI increment effect $F_{2,22} = 0.07$, both p 's > 0.050) for this parameter. No further differences were found between groups for the other parameters: percentage correct responding when the ITI duration was reduced (genotype effect $F_{1,11} = 0.79$; genotype x ITI reduction effect $F_{2,22} = 0.55$) or increased (genotype effect $F_{1,11} =$

0.79, genotype x ITI increment effect $F_{2,22} = 0.55$), reinforcers earned when the ITI duration was reduced (genotype effect $F_{1,11} = 0.56$, genotype x ITI reduction effect $F_{2,22} = 0.76$) or increased (genotype effect $F_{1,11} = 0.44$; genotype x ITI increment effect $F_{2,22} = 1.72$), errors of omission when the ITI duration was reduced (genotype effect $F_{1,11} = 1.41$; genotype x ITI reduction effect $F_{2,22} = 2.34$) or increased (genotype effect $F_{1,11} = 0.28$; genotype x ITI increment effect $F_{2,22} = 2.35$), incorrect response latency (genotype effect $F_{1,11} = 0.19$; genotype x ITI reduction effect $F_{2,22} = 2.14$) or increment (genotype effect $F_{1,11} = 0.08$; genotype x ITI increment effect $F_{2,22} = 0.84$) and biased responding during ITI duration reduction (genotype effect $F_{1,11} = 0.69$; genotype x ITI reduction effect $F_{2,22} = 0.18$) or increment (genotype effect $F_{1,11} = 0.00$; genotype x ITI increment effect $F_{2,22} = 1.22$) (all p 's > 0.05).

Effects of social defeat

Mice were exposed to a social defeat procedure prior to testing. No effects on performance were found: percentage correct responding ($F_{1,11} = 0.04$), reinforcers earned ($F_{1,11} = 0.86$), errors of omission ($F_{1,11} = 1.72$), correct response latency ($F_{1,11} = 0.00$), incorrect latency ($F_{1,11} = 3.03$), biased responding ($F_{1,11} = 0.43$), and ITI responding ($F_{1,11} = 4.40$) (all p 's > 0.05).

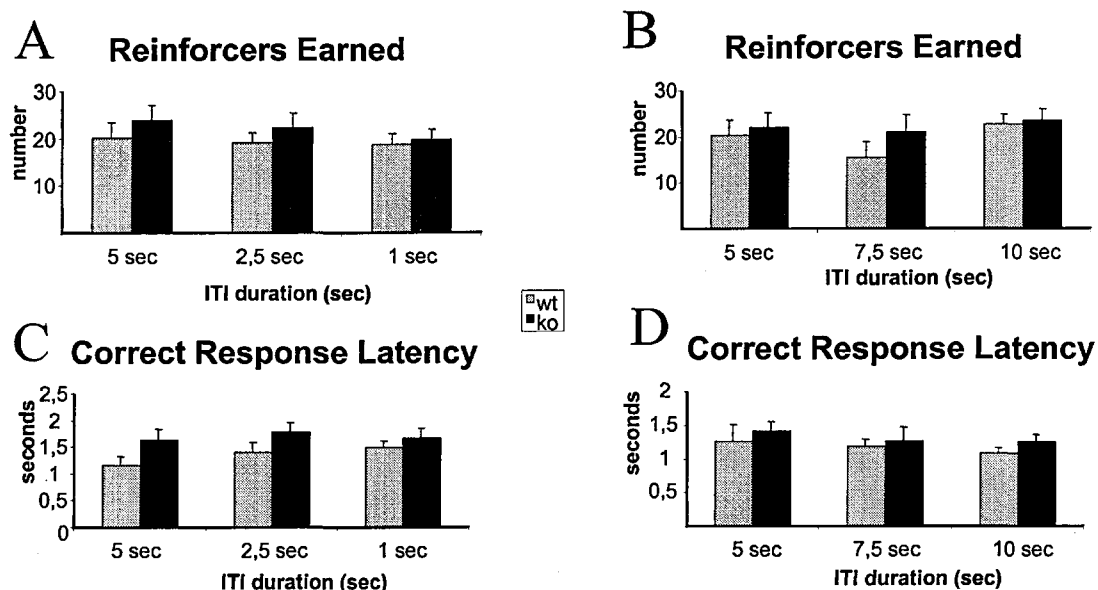


Figure 9.5 Effects of alteration of ITI duration on 5-CSRTT performance in mice lacking CRH. Treatment given in ascending order. (A) number of reinforcers earned, reduction of ITI duration (B) number of reinforcers earned increment of ITI duration, (C) correct response latency, reduction of ITI duration (D) correct response latency, increment of ITI duration. Data are presented as means, with error bars denoting SEMs.

No differences depending on genotype were found in this experimental stage; percentage correct responding (genotype effect $F_{1,11} = 0.50$; social defeat x genotype effect $F_{1,11} = 0.13$), reinforcers earned (genotype effect $F_{1,11} = 1.07$, social defeat x genotype effect $F_{1,11} = 0.65$), errors of omission (genotype effect $F_{1,11} = 1.16$; social defeat x genotype effect $F_{1,11} = 0.59$), correct response latency (genotype effect $F_{1,11} = 0.12$; social defeat x genotype effect $F_{1,11} = 2.25$), incorrect latency (genotype effect $F_{1,11} = 0.94$; social defeat x genotype effect $F_{1,11} = 0.13$), biased responding (genotype effect $F_{1,11} = 1.25$; social defeat x genotype effect $F_{1,11} = 0.74$), and ITI responses (genotype effect $F_{1,11} = 0.17$; social defeat x genotype effect $F_{1,11} = 0.06$) (all p 's > 0.050 ; Table 9.2).

Table 9.2 Effect of social defeat in mice lacking CRH on 5-CSRTT performance (data are expressed as means \pm SEMs).

Measure	wildtype basal	Knockout Basal	wildtype social defeat	Knockout social defeat
No. Reinforcers	14.00 \pm 3.43	17.83 \pm 3.44	14.14 \pm 3.51	19.83 \pm 2.76
Earned				
% Correct Responses	50.67 \pm 3.86	53.66 \pm 4.67	50.20 \pm 5.60	55.36 \pm 3.62
Errors of Omission	33.57 \pm 4.58	27.23 \pm 5.84	32.57 \pm 5.06	23.50 \pm 5.33
No. ITI Responses	0.11 \pm 0.02	0.12 \pm 0,03	0.09 \pm 0.01	0.10 \pm 0.02
Corr. Resp. Lat. (sec)	1.20 \pm 0.14	1.37 \pm 0.15	1.41 \pm 0.13	1.15 \pm 0.11
Incorr. Resp. Lat.	4.11 \pm 0.50	3.62 \pm 0.19	3.52 \pm 0.30	3.25 \pm 0.36
(sec)				
Bias (Index Y)	0.37 \pm 0.08	0.21 \pm 0.06	0.27 \pm 0.09	0.23 \pm 0.09

All p's > 0.050; No.: number; Corr. Resp. Lat.: correct response latency; Incorr. Resp. Lat.: incorrect response latency

Effect of Forced swimming on 5-CSRRT

Although forced swimming did not affect percentage correct ($F_{1,11} = 0.06$, $p > 0.05$), the number of reinforcers earned was significantly increased ($F_{1,11} = 12.37$, $p = 0.005$), and concomitantly, the errors of omission were reduced ($F_{1,11} = 12.16$, $p = 0.005$) (Figures 9.6A, B, C). No further forced swimming-induced differences were found in correct response latency ($F_{1,11} = 2.70$), incorrect latency ($F_{1,11} = 0.28$), biased responding ($F_{1,11} = 1.06$) or ITI responses ($F_{1,11} = 1.05$) (all p's > 0.050).

Forced swimming did not induce any genotype-dependent differences: percentage correct responding (genotype effect $F_{1,11} = 0.11$; forced swimming x genotype effect $F_{1,11} = 0.13$), reinforcers earned (genotype effect $F_{1,11} = 1.43$; forced swimming x genotype effect $F_{1,11} = 0.03$), errors of omission (genotype effect $F_{1,11} = 1.67$; forced swimming x genotype effect $F_{1,11} = 0.44$), correct response latency (genotype effect $F_{1,11} = 1.16$; forced swimming x genotype effect $F_{1,11} = 2.57$), incorrect latency (genotype effect $F_{1,11} = 0.03$; forced swimming x genotype effect $F_{1,11} = 0.59$), biased responding (genotype effect $F_{1,11} = 0.21$; forced swimming x genotype effect $F_{1,11} = 3.35$), and ITI responding (genotype effect $F_{1,11} = 3.77$; forced swimming x genotype effect $F_{1,11} = 0.77$) (all p 's > 0.050).

Effect of acute scopolamine treatment

There was an overall effect of treatment on percentage correct responding, with scopolamine leading to reduced accuracy ($F_{4,40} = 5.44$, $p = 0.026$) (Figure 9.7B). This effect was independent of genotype (genotype effect $F_{1,10} = 1.25$; genotype x treatment effect $F_{4,40} = 0.38$, both p 's > 0.050). Likewise, scopolamine reduced the number of reinforcers earned (treatment effect $F_{4,40} = 41.45$, $p < 0.001$). Although no genotype x treatment interaction was found (treatment x genotype effect $F_{4,40} = 1.03$, $p > 0.05$), CRH knockout animals earned overall more reinforcers compared to wildtype littermates (genotype effect $F_{1,10} = 13.89$, $p = 0.004$) (Figure

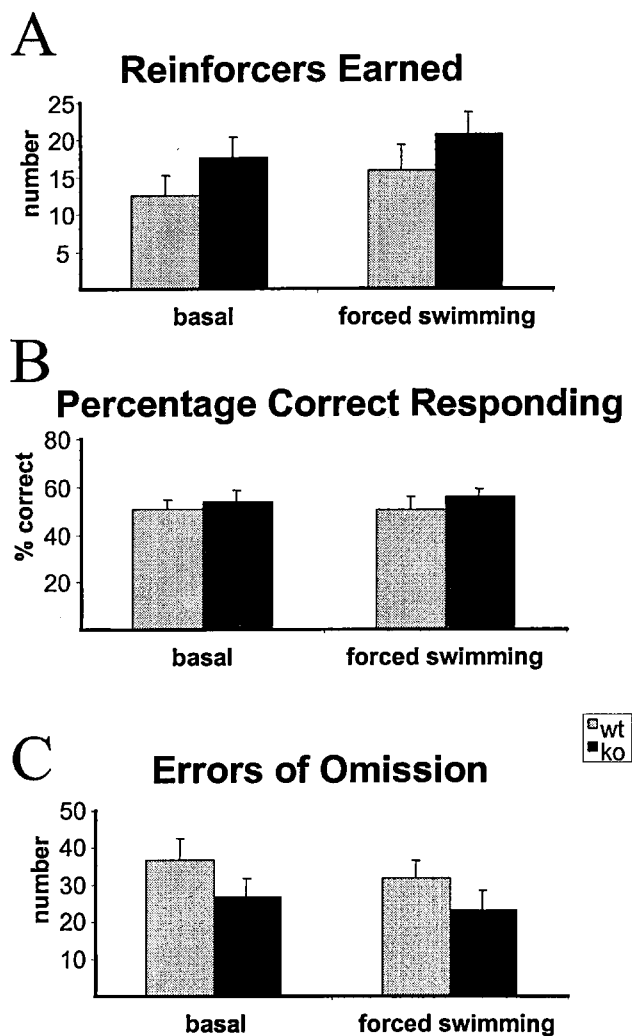


Figure 9.6 Effects of forced swimming 5-CSRTT in mice lacking CRH. (A) number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission. Data are presented as means, with error bars denoting SEMs.

9.7A). Scopolamine treatment resulted in an increase of errors of omission ($F_{4,40} = 96.26$, $p < 0.001$). Again no genotype x treatment interaction was found ($F_{4,40} = 0.46$, $p > 0.05$), but CRH knockout animals were less disrupted by treatment in terms of overall reduced errors of omission ($F_{1,10} = 5.08$, $p < 0.048$) (Figure 9.7C). Correct response latency was not affected by treatment ($F_{4,40} = 0.86$, $p > 0.05$), and no difference depending on genotype was found (genotype effect $F_{1,10} = 0.19$; treatment

x genotype effect $F_{4,40} = 1.90$, both p 's > 0.050). Furthermore, no effect of treatment was found in terms of incorrect response latency (treatment effect $F_{4,40} = 2.48$; treatment x genotype effect $F_{4,40} = 0.44$, both p 's > 0.050). Strikingly, a genotype-dependent effect was found for this parameter, just reaching significance ($F_{1,10} = 5.14$, $p = 0.049$; Figure 9.7D). Visual inspection revealed that the group difference varied between dosages, but more or less at random. Scopolamine also affected the ITI responses made ($F_{4,40} = 10.49$, $p = 0.004$), whereas no genotype-dependent effect was found (genotype effect $F_{1,10} = 0.14$; treatment x genotype effect $F_{4,40} = 0.34$, both p 's > 0.050) (Figure 9.7D). Furthermore, scopolamine treatment had no effect on biased responding (treatment effect $F_{4,40} = 0.66$; treatment x genotype effect $F_{4,40} = 1.15$, both p 's > 0.050), but again a genotype effect was found ($F_{1,10} = 8.74$, $p = 0.016$). Visual inspection revealed that wildtype animals showed increased biased responding (Figure 9.7F).

Discussion

Both groups performed equally well during autoshaping and simple visual discrimination learning, with exception of a mild decrease of biased responding of CRH knockout mice, suggesting that lack of CRH does not affect learning capacity in this task. Although no effect of session was found during the autoshaping procedure, both groups performed well above chance from the first session of the

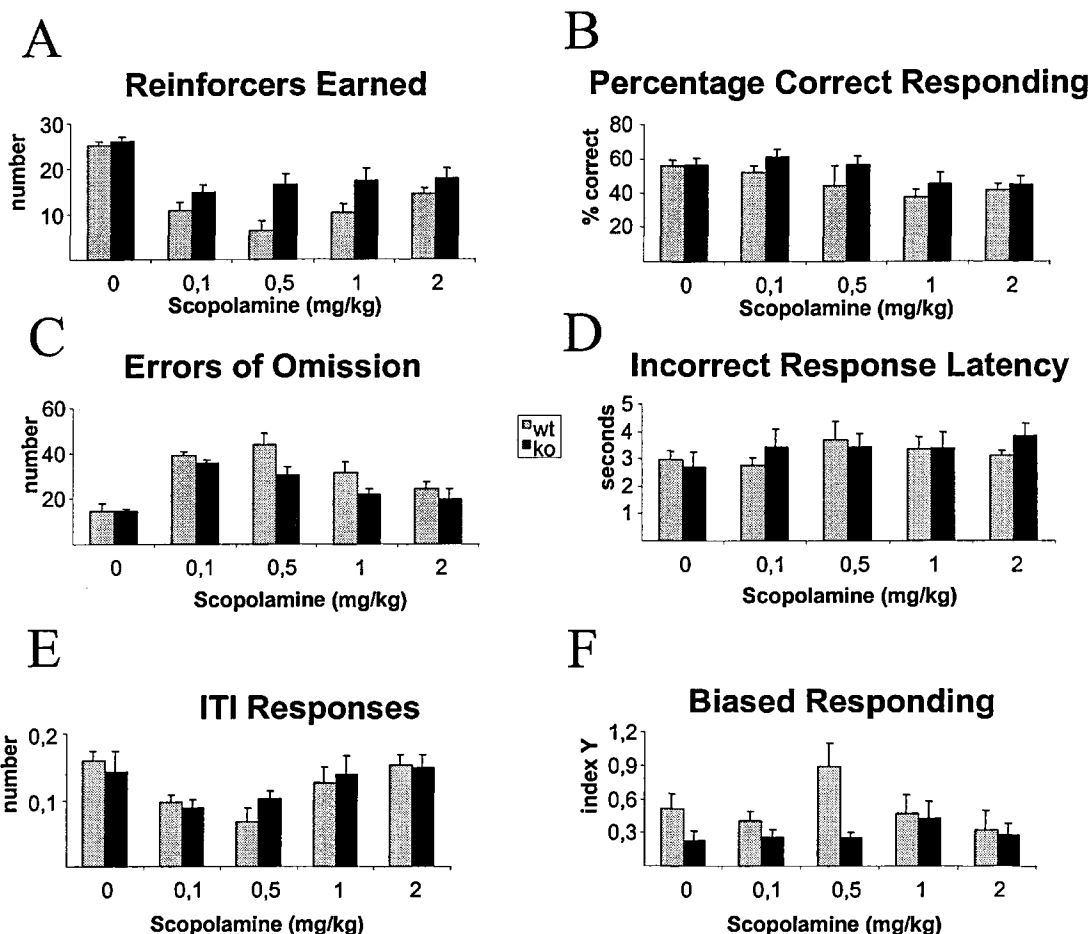


Figure 9.7 Effects of scopolamine on 5-CSRTT in mice lacking CRH. Scopolamine was administered at dosages of 0.0, 0.1, 0.5 1.0 and 2.0 mg/kg i.p., 20 min prior to testing. Treatment was given in ascending order. (A) number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) incorrect response latency, (E) ITI responses per trial (F) Biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

simple visual discrimination learning phase onwards, suggesting animals learned the association between the stimulus and reward. By reducing the stimulus duration, reinforcers earned and percentage correct responding were decreased. Although the attentional load was increasing, less errors of omission were made, and both correct and incorrect response latencies decreased, whilst biased responding increased. Both groups performed still equally well during this phase. Furthermore, when animals

were tested for an extended period of 20 sessions with shortest stimulus duration (0.5 s), no differences depending on genotype were found, suggesting that lack of CRH does not affect visuospatial attention under these testing circumstances.

It has been demonstrated that effects of manipulations can depend on the attentional load. For example, rats with ceruleo-cortical noradrenaline loss perform equally well compared to control animals under basal conditions, whereas lesioned animals were impaired in presence of white noise in terms of accurate responding (Carli et al., 1983). Presentation of white noise did not induce group difference. In fact, it resulted in an increase in percentage correct responding and in errors of omission, without affecting the number of reinforcers earned. Altering ITI duration had no clear effects on performance in both groups of animals.

The CRH system is activated after stressors. Therefore it was investigated whether performance was differently affected by stressors. Data from this chapter and chapter 8 indicate that the effects of stress before testing depends on the kind of stressor to which the animals are exposed. Thus, i.p. saline injection increased accurate responding, but in the same time it reduced reinforcers earned, and increased errors of omission made. In other words, animals showed a stronger tendency to refrain from responding, which consequently led to a net decrease in reinforcers earned but when animals responded, they responded more accurate.

In the present study, the effect of lack of CRH on 5-CSRTT performance after two distinct types of stressors was investigated: physical/physiological stress (inescapable swim stress) and psychological stress (social defeat). Social defeat failed to affect

performance, whereas forced swim stress prior to testing resulted in an increase in reinforcers earned, a decrease in errors of omission, but leaving parameters that reflect attentional performance unaffected. Therefore, swim stress seemed to affect non-specific factors. This indicates that performance in the 5-CSRTT is a robust phenomenon and those physical/physiological and psychological stressors have distinct effect on performance. Furthermore, no differences between groups were found after exposure to stress, this in contrast to the impairments on performance in mice overproducing CRH (Chapter 9). Stressful stimuli have been shown to increase the apparent release of CRH in the central amygdaloid nucleus (Pich et al., 1993), and it has been demonstrated that social defeat evoked an elevation of CRH in the hypothalamus in rats (Skutella et al 1994). This suggests that long-term hyperactivity of the CRH system reduces sustained and divided attention, whereas acute CRH increase does not. However, it cannot be ruled out that inescapable swim stress or social defeat leads to an inadequate level of stress after, and that therefore CRH levels were not adequately high. This is unlikely because inescapable swim stress strongly activates HPA-axis activity that is CRHR1 mediated (Timple et al., 1998). Alternatively, it is possible that CRH disrupts attentional processes after long-term hyperactivity.

Muscaric receptor blockade resulted in disruption in performance as indicated by the reduction in accuracy and reinforcers earned and an increase of errors of omission made, in a similar fashion as presented in Chapter 8. Strikingly, the disruptive effects of scopolamine treatment were stronger in wildtype animals compared to knockout littermates. This indicates again that long-term of the CRH system affects other neurotransmitter system.

In summary, lack of CRH does not result in altered sustained and divided attention. In addition, exposure to stress does not result in genotype dependent effects, suggesting that, in contrast to the disturbing effects on attentional process after long-term excess of CRH described in Chapter 9, acute increase in CRH activity does not affect attentional function. However, it is possible that lack of CRH could be compensated by other ligands of the CRH peptide family, for example UCN, UCNII or UCNIII, which also stimulate CRH receptors. Therefore, investigation of the role of CRHR1 and CRHR2 is necessary to fully understand the role of CRH in attention.

Chapter 10

Effects of reduced CRHR1 activity on five-choice serial reaction time performance in mice

Introduction

The experiments presented in Chapter 8 indicated that chronic CRH excess may disrupt visuospatial attentional processes as measured in transgenic mice overproducing CRH, performing on an operant 5-CSRTT. In contrast lack of CRH did no result in any disturbance in these processes (Chapter 9). However, CRH is not the only ligand that stimulates the CRH receptors. To further elucidate the possibility that these effects are mediated via CRHR1 activation, the effects of acute blockade or life-long loss of CRHR1 was studied. In a first experiment, the effects of acute administration of a non-peptidergic, selective CRHR1 antagonist (R121919) were tested, with and without concomitant cholinergic blockade by the muscarinic antagonist scopolamine.

R121919 is a 3-phenyl-pyrazolo-pyrimidine with high affinity to human and rat CRHR1 ($K_i = 3.15$ nmol/l). R121919 has been reported to reduce ^{125}I -oCRH binding density in brain regions expressing CRHR1, while leaving binding to CRHR2 unaffected (Keck et al., 2001). Oral bioavailability is 10 – 20% in rats (Wilcoxon et al., 2000). Ex vivo, R121919 occupies approximately 50% of CRHR1 in rat cortex one h after an oral dose of 2.5 mg/kg, while a 20 mg/kg dose results in almost a complete receptor occupancy (Langlois and Jurzak, Janssen Research Foundation unpublished results). EC_{50} for inhibition of CRH-stimulated ACTH release has been reported to be at 20 nmol/l (Wilcoxon et al., 2000). Furthermore, systemic administration of R121919 blunts the responsivity of the HPA axis following exposure to mild stress and has anxiolytic properties in rats bred for increased anxiety-related behaviour (Keck et al., 2001). The effects of acute administration of R121919 were tested over a wide dose range and the interaction of this compound with cholinergic blockade by the muscarinic antagonist scopolamine was studied.

In a second experiment, the effects of long-term loss of CRHR1 on visuospatial attention was studied in mice deficient for CRHR1 performing the 5CSRTT. These mutants exhibit increased exploratory activity and reduced anxiety-related behaviour (Timpl et al., 1998). It remains a possibility that these behavioural alterations are, at least in part, a consequence of altered arousal and/or attention directed towards contextual cues in these animals.

Material and methods

Animals

Two months old male C57BL/6Cr1BR mice were purchased from Charles River, Sulzfeld, Germany for the first experiment. These animals were first trained to stable baseline. Mice were between four and six months old at the time of testing. For the second experiment, eight two-months-old male homozygous *Crhr1*^{-/-} mutants and thirteen littermate wildtype (+/+) mice were generated by intercross from 129/Ola × CD-1 F1 hybrids (Timpl et al., 1998). This CRHR1-deficient mouse line was established by deleting the coding sequences of the transmembrane regions V, VI and VII, including the G-coupling domain and the intracellular cytoplasmatic tail. This results in a truncated protein unable to activate the adenylate cyclase second messenger system or to transmit any ligand-induced signals. In both experiments, animals were housed individually and maintained on a 12:12 h light/dark cycle (lights on at 06:00 hours) with *ad libitum* water throughout. They were maintained at 85-90% of their free feeding weight, starting one week prior to the beginning of the experiment.

5-CSRRT

Testing took place during the light phase of the light/dark cycle. Five sessions were scheduled per week (one session per day, Monday-Friday). Animals were trained as described in detail in Chapter 2. In brief, animals were habituated the testing apparatus until they reliably ate all the pellets presented (2 per hole), after which they were trained on the autoshaping procedure. Seven sessions were scheduled during this experimental stage. This was followed by training the animals following the

visual discrimination learning protocol until stable performance was reached. Next, the stimulus duration was gradually reduced (i.e., 8 s, 4 s, 2 s, 1 s, 0.5 s), with 5 sessions per stimulus duration.

Effects of R121919 on baseline schedule

Once baseline responding was established at 0.5 s stimulus duration, 5 sessions were scheduled where animals received i.p. saline injections prior to test. R121919 (kindly donated by Janssen Research Foundation, Beerse, Belgium) was dissolved in vehicle (3 % Tween 20 in saline) and injected i.p. in a volume of 10 ml/kg, 20 min before testing (0.0, 0.5, 1.0, 2.5, 10.0, 20.0, 40 mg/kg). Treatment was given in ascending order. At least two days lapsed in-between drug sessions in order to allow for sufficient washout of the drug.

Effects of R121919 in the presence of white noise

Subsequently, the effects of CRHR1 blockade in the presence of a distractor (a white noise stimulus (1.2 kHz, 76 dB), presented during the 500 ms preceding the stimulus onset, were studied. Only the high dose (40 mg/kg) versus vehicle was tested.

Effects of scopolamine on baseline performance

Scopolamine hydrochloride (Sigma Chemicals, Deisenhofen, Germany) was dissolved in saline and injected i.p. in a volume of 10 ml/kg, 20 min before testing (0.0, 0.1, 0.5, 1.0 mg/kg). Again, treatment was given in ascending order, and at least two days lapsed in-between drug sessions.

Interaction between R121919 and scopolamine

Finally, animals received concomitant treatment with both scopolamine (0.5 mg/kg i.p., 20 min prior to test) and R121919 (40 mg/kg, i.p., 20 min prior to test). Drugs were administered according to a Latin square design and animals were tested under baseline conditions.

Effects of CRHR1 knockout on task acquisition

Autoshaping was analysed in CRHR1 knockout mice in the second experiment. This was followed by training animals on simple discrimination with stimuli of long duration (8 s). Subsequently, the effects of gradually reducing stimulus duration was investigated. Animals were tested on shortest stimulus duration for 14 sessions before proceeding to the next stage.

Effects of CRHR1 knockout under conditions of altered stimulus predictability

Temporal predictability of the stimulus was altered during six sessions by introduction of ITIs of various duration, first three sessions during which ITI durations were shortened (5, 2 and 1 s), followed by three sessions with longer ITIs (ranging from 5, 10, to 20 s).

Results

Effects of acute administration of R121919

R121919, in the dose range tested, failed to affect accuracy in terms of percentage correct responses ($F_{6,72} = 0.62$, $p > 0.05$) (Figure 10.1B). However, there was a

tendency for a reduction in the number of reinforcers earned with higher doses ($F_{6,72} = 2.31$, $p = 0.067$), and a concomitant increase in errors of omission was observed ($F_{6,72} = 4.12$, $p = 0.009$) (Figures 10.1A, C). Further analysis revealed that animals made significantly more errors of omission with doses of 10.0 mg/kg and higher when compared to vehicle. Treatment had no significant effect on ITI responses ($F_{6,72} = 0.31$), correct or incorrect response latencies ($F_{6,72} = 1.11$ and $F_{6,72} = 1.17$, respectively), or biased responding ($F_{6,72} = 0.83$; all p 's > 0.050) (Figures 10.1D-G).

Effects of acute administration of R121919 in the presence of white noise

The high-dose of R121919 also failed to affect percentage correct responses in the presence of white noise ($F_{1,12} = 0.11$, $p > 0.05$) (Figure 10.2B). However, the difference between vehicle treatment and treatment with 40 mg/kg R121919 now reached significance in terms of the number of reinforcers earned ($F_{1,12} = 5.90$, $p = 0.032$) (Figure 10.2A). There was also a small effect on the errors of omission measure, which just about reached significance ($F_{1,12} = 4.76$, $p = 0.050$) (Figure 10.2C). Again, treatment had no effect on ITI responses ($F_{1,12} = 1.01$, $p > 0.05$) or biased responding ($F_{1,12} = 0.06$, $p > 0.05$) and left correct response latencies unaffected ($F_{1,12} = 0.30$, $p > 0.050$) (Figure 10.2D, E, G). However, there was an effect of treatment on incorrect response latency ($F_{1,12} = 6.80$, $p = 0.023$), with shortened latency following treatment with R121919 (Figure 10.2F).

Effects of acute treatment with scopolamine

In contrast to R121919, scopolamine induced a dose-dependent accuracy deficit ($F_{3,33} = 15.85$, $p < 0.001$), with both the 0.5 mg/kg and the 1.0 mg/kg dose impairing percentage correct responding when compared to animals treated with saline or the

lowest dose (0.1 mg/kg) of the drug (Figure 10.3B). Furthermore, a dose-dependent reduction in the number of reinforcers earned ($F_{3,33} = 43.23$, $p < 0.001$) and a concomitant increase in errors of omission ($F_{3,33} = 14.45$, $p < 0.001$) was observed (Figures 10.3A, C). ITI responses were just about significantly decreased at the lowest dose of scopolamine tested (0.1 mg/kg), but not with higher doses ($F_{3,33} = 3.22$, $p = 0.049$) (Figure 10.3D). However, inspection of the data suggested that this effect was primarily due to a change in baseline under saline treatment as vehicle-treated animals in general performed at lower levels throughout the other stages of the study (cf. Figures 10.1D, 10.2D, 10.4D). Scopolamine failed to significantly alter correct ($F_{3,33} = 1.71$, $p > 0.05$) or incorrect ($F_{3,33} = 0.94$, $p > 0.05$) response latencies (Figures 10.3E, F), but a tendency for an increase in biased responding was noted ($F_{3,33} = 3.37$, $p = 0.059$) (Figure 10.3G).

Interaction between R121919 and scopolamine

There was a main effect of treatment with scopolamine which, at a dose of 0.5 mg/kg, again led to a decrease in accurate performance ($F_{1,11} = 42.01$, $p < 0.001$). However, there was no effect of R121919 (40 mg/kg) ($F_{1,11} = 0.42$) and no interaction between the two compounds was observed ($F_{1,11} = 1.47$; both $p > 0.05$; Fig. 4B). In contrast, both scopolamine ($F_{1,11} = 10.58$, $p = 0.008$) and R121919 ($F_{1,11} = 12.76$, $p = 0.004$) led to a decrease in the number of reinforcers earned. The effect of combined treatment was additive rather than potentiating, as indicated by the lack of significant interaction ($F_{1,11} = 0.002$, $p > 0.05$; Figure 10.4A).

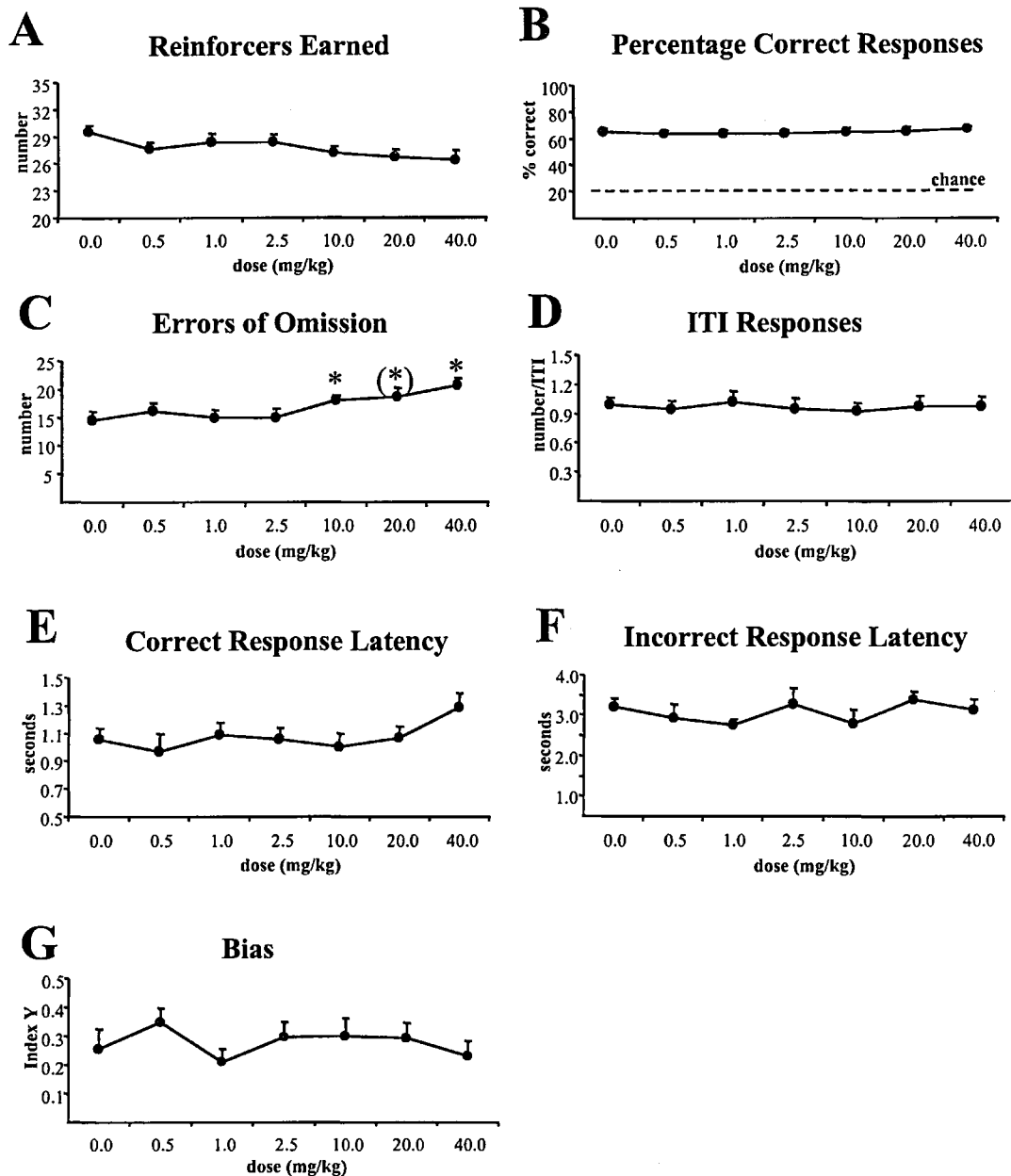


Figure 10.1 Effects of CRHR1 antagonism with R121919 on 5-CSRTT performance. (A) reinforcers earned, (B) percentage correct responses (C) errors of omission (D) ITI responses (E) correct response latency, (F) Incorrect response latency, (G) biased responding. *: significant difference to vehicle, *: $p < 0.05$, (*): $p = 0.051$ (data are expressed as means and SEMs).

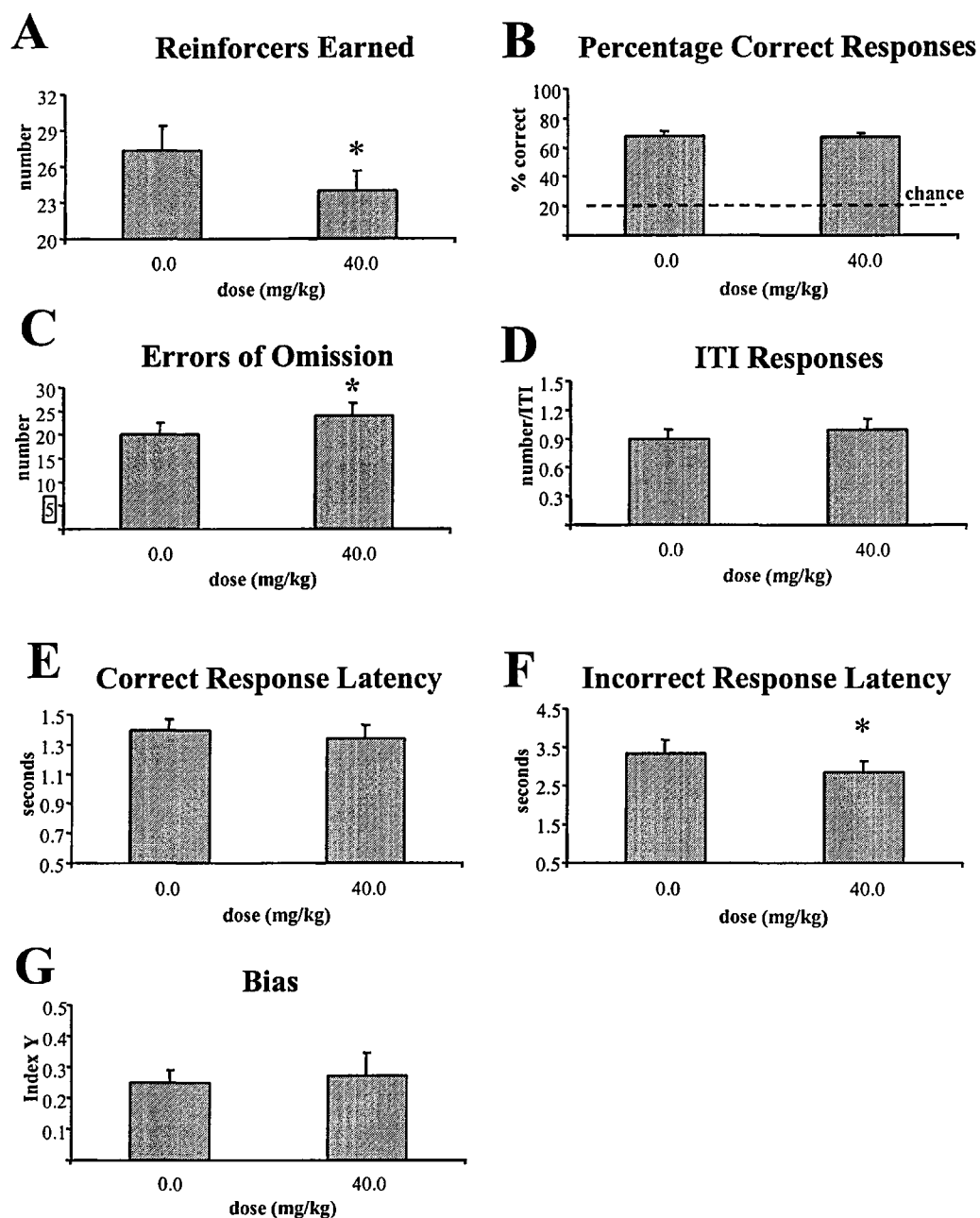


Figure 10.2 Effects of CRHR1 antagonism with R121919 on 5-CSRTT performance in the presence of white noise, (A) number of reinforcers earned, (B) percentage correct responding, (C) errors of omission, (D) ITI responses, (E) correct response latency, (F) incorrect response latency, (G) biased responding; *: significant difference to vehicle, $p < 0.05$ (data are expressed as means values and SEMs).

Errors of omission were increased following administration of the CRHR1 antagonist ($F_{1,11} = 11.66$, $p = 0.006$), while scopolamine alone ($F_{1,11} = 0.19$, $p > 0.05$) or in combination ($F_{1,11} = 0.07$, $p > 0.05$) had no additional effect (Figure 10.4C). Moreover, an increase in ITI responses was seen following administration of scopolamine only ($F_{1,11} = 7.12$, $p = 0.022$; Figure 10.4D), while R121919 ($F_{1,11} = 2.79$) or its combination with scopolamine ($F_{1,11} = 1.01$; both p 's > 0.050) had no additional effect on this measure (Figure 10.4D). In addition, scopolamine increased correct ($F_{1,11} = 5.83$, $p = 0.034$), but not incorrect ($F_{1,11} = 0.24$, $p > 0.05$) response latencies (Figures 10.4E, F). A tendency for R121919 to increase correct response latencies ($F_{1,11} = 3.92$, $p = 0.073$) was also noted, while blockade of CRHR1 had no effect on incorrect latency ($F_{1,11} = 1.70$, $p > 0.05$). However, the interaction between scopolamine and R121919 reached significance for the correct response latency measure ($F_{1,11} = 8.33$, $p = 0.015$), indicating that combined treatment was more effective than single treatment to increase response latencies (Figure 10.4E). Incorrect response latency was not significantly altered by the drug combination, but a tendency was observed ($F_{1,11} = 3.55$, $p = 0.086$). Finally, bias remained unaffected by all drug combinations (scopolamine effect $F_{1,11} = 0.74$; R121919 effect $F_{1,11} = 0.007$; scopolamine \times R121919 effect $F_{1,11} = 0.27$; all p 's > 0.050 ; Figure 10.4G).

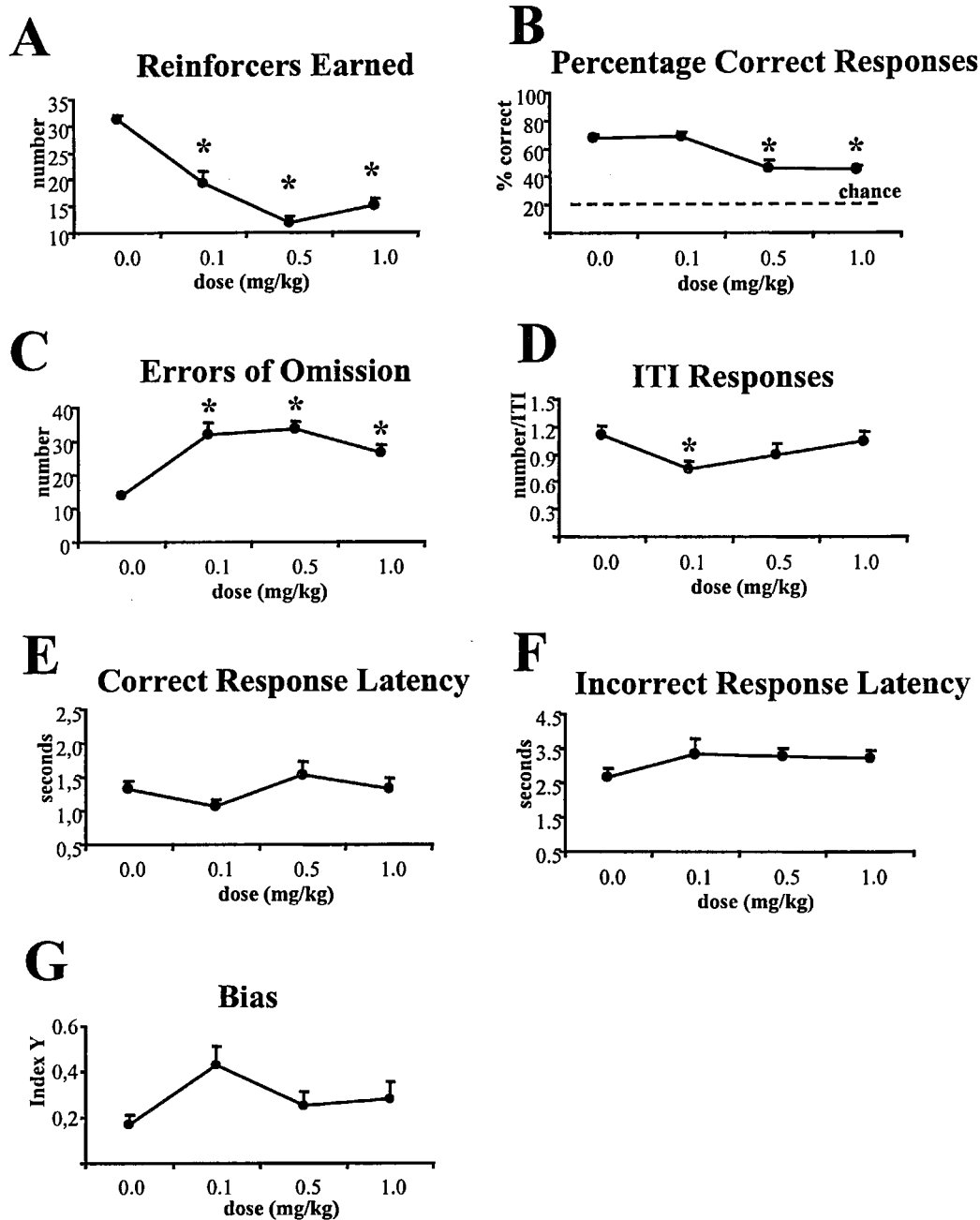


Figure 10.3 Effects of scopolamine on 5-CSRTT performance, (A) number of reinforcers earned, (B) percentage correct responding, (C) errors of omission, (D) ITI responses, (E) correct response latency, (F) incorrect response latency, (G) biased responding; *: significant difference to vehicle, $p < 0.05$ (data are expressed as means and SEMs).

Autoshaping in CRHR1 knockout mice

All animals completed the 20 trials scheduled per session within the time limit of 30 min. Groups did not differ in the number of responses made until the illuminated (correct) hole was visited (genotype effect $F_{1,19} = 1.50$; genotype \times session effect $F_{6,114} = 1.69$, both p 's > 0.050 ; Figure 10.5A), in the latency to complete a trial (genotype effect $F_{1,19} = 0.44$; genotype \times session effect $F_{6,114} = 1.38$, both p 's > 0.050 ; data not shown), in the relative number of trials completed within the limited hold of 5 s, i.e., prior to the automatic delivery of a pellet (genotype effect $F_{1,19} = 2.72$; genotype \times session effect $F_{6,114} = 0.48$, both p 's > 0.050 ; Figure 10.5B) or in the number of nose-pokes made during the ITI (genotype effect $F_{1,19} = 2.61$; genotype \times session effect $F_{6,114} = 1.28$, both p 's > 0.050 ; Figure 10.5C).

Simple visual discrimination learning in CRHR1 knockout mice

During the initial stage of visual discrimination, the stimulus was presented for maximally 8 s) No differences were observed between groups in the number of reinforcers earned (genotype effect $F_{1,17} = 0.67$; genotype \times session effect $F_{7,119} = 1.74$, both p 's > 0.050), percentage correct responding (genotype effect $F_{1,17} = 0.08$; genotype \times session effect $F_{7,119} = 2.01$, both p 's > 0.050), or errors of omission (genotype effect $F_{1,17} = 2.77$; genotype \times session effect $F_{7,119} = 0.79$, both p 's > 0.050) (Figures. 10.6A – C). Likewise, there was no difference in the number of ITI responses (genotype effect $F_{1,17} < 0.01$; genotype \times session effect $F_{7,119} = 0.44$, both p 's > 0.050), correct (genotype effect $F_{1,17} = 0.06$; genotype \times session effect $F_{7,119} = 1.14$, both p 's > 0.050) or incorrect (genotype effect $F_{1,17} = 3.36$; genotype \times session

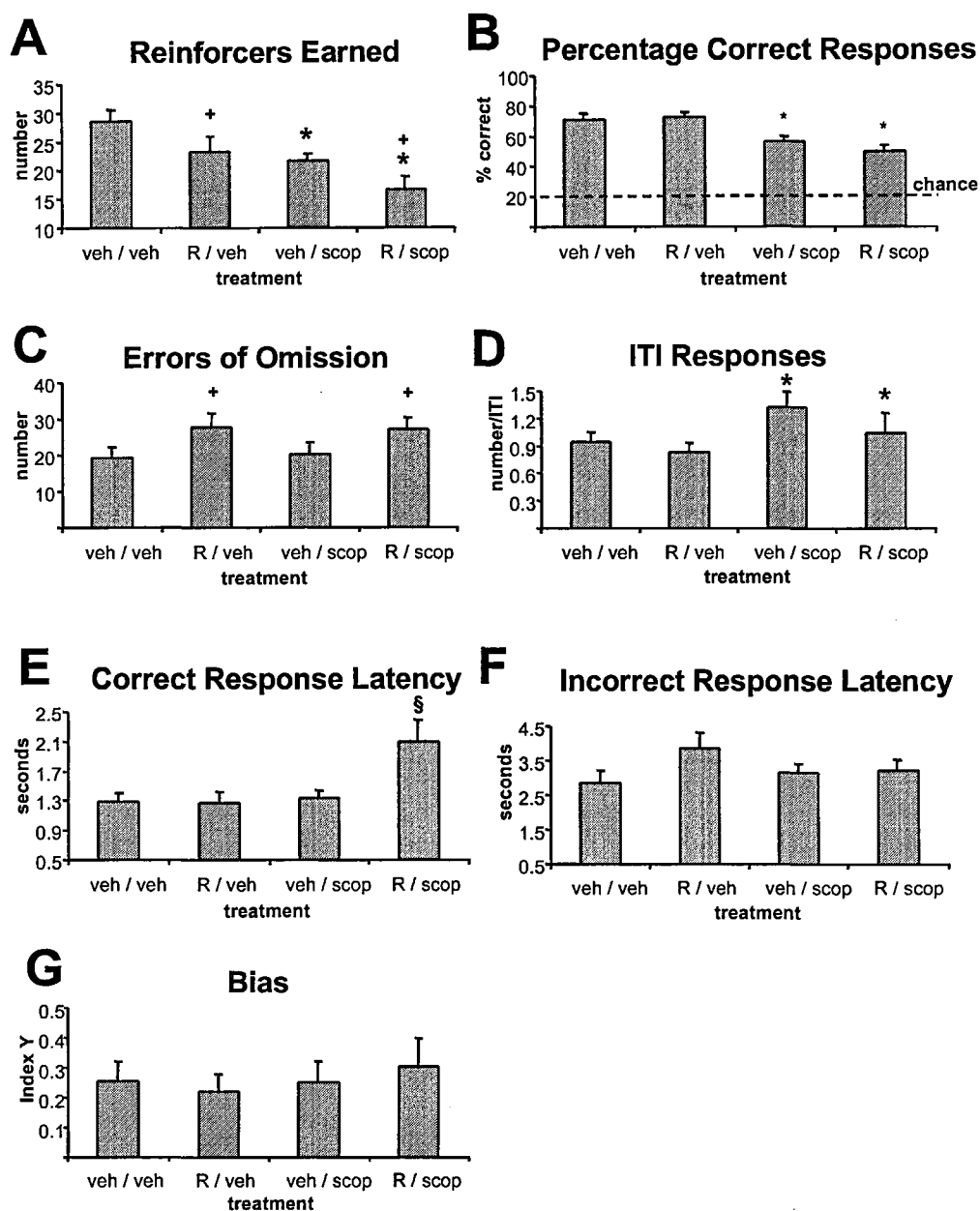


Figure 10.4 Interaction between R121919 (40.0 mg/kg) and scopolamine (0.5 mg/kg) on 5-CSRTT performance, (A) number of reinforcers earned, (B) percentage correct responding, (C) errors of omission, (D) ITI responses, (E) correct response latency, (F) incorrect response latency, (G) biased responding; *: significant effect of scopolamine, $p < 0.05$; +: significant effect of R121919, $p < 0.05$; §: significant interaction, $p < 0.05$ (data are expressed as means and SEMs).

effect $F_{7,119} = 0.77$, both p 's > 0.050) response latencies, or biased responding (genotype effect $F_{1,17} = 0.47$; genotype \times session effect $F_{7,119} = 0.79$, both p 's > 0.050 ; Figures 10.6D – G). Of note, both groups already performed well above chance during the first discrimination session, indicating that animals already acquired the association between light stimulus and reinforcement during the autoshaping stage.

Effects of reduced stimulus duration on performance of CRHR1 knockout mice

The number of reinforcers earned (stimulus reduction effect $F_{3,54} = 23.29$, $p < 0.001$), percentage correct responses (stimulus reduction effect $F_{3,54} = 52.73$, $p < 0.001$), errors of omission (stimulus reduction effect $F_{3,54} = 4.53$, $p = 0.017$), correct (stimulus reduction effect $F_{3,54} = 76.82$, $p < 0.001$) and incorrect (stimulus reduction effect $F_{3,54} = 10.49$, $p < 0.001$) response latencies all declined with decreasing stimulus duration (Figures 10.7A – C, E, F), while the number of responses made during the ITI remained unaffected (stimulus reduction effect $F_{3,54} = 2.22$, $p > 0.05$; Figure 10.7D). Biased responding, on the other hand, increased with decreasing stimulus duration (stimulus reduction effect $F_{3,54} = 10.22$, $p < 0.001$; Figure 10.7G).

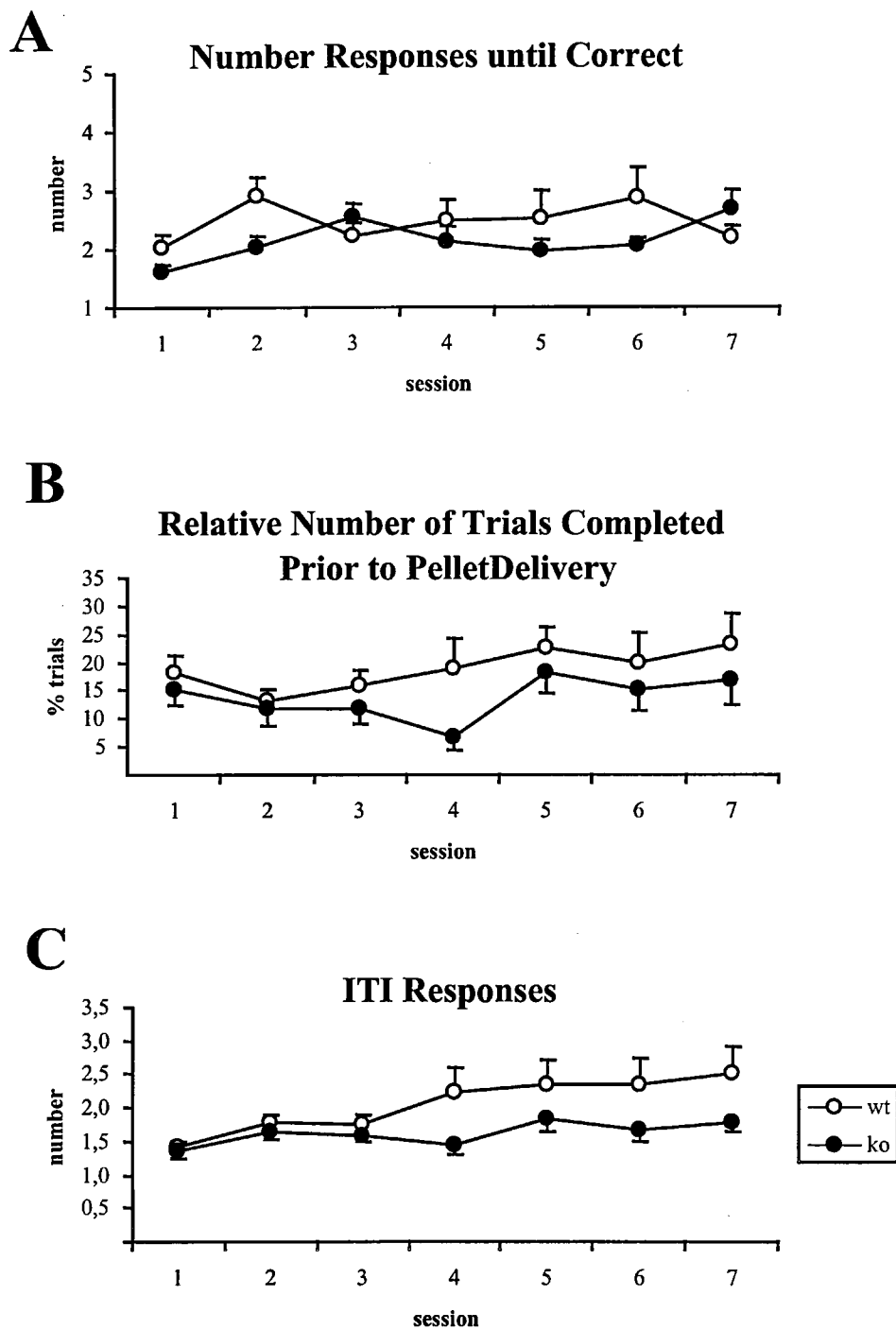


Figure 10.5 Effects of lack of CRHR1 on autoshaping, wildtype mice (wt), CRHR1^{-/-} mutant (ko). (A) number of responses made until a nose poke into the illuminated hole was performed, (B), relative number of trials completed prior to pellet delivery, (C) number of responses made during the ITI. wt: wildtype control, ko: CRHR1^{-/-} mutant (data are expressed as means and SEMs).

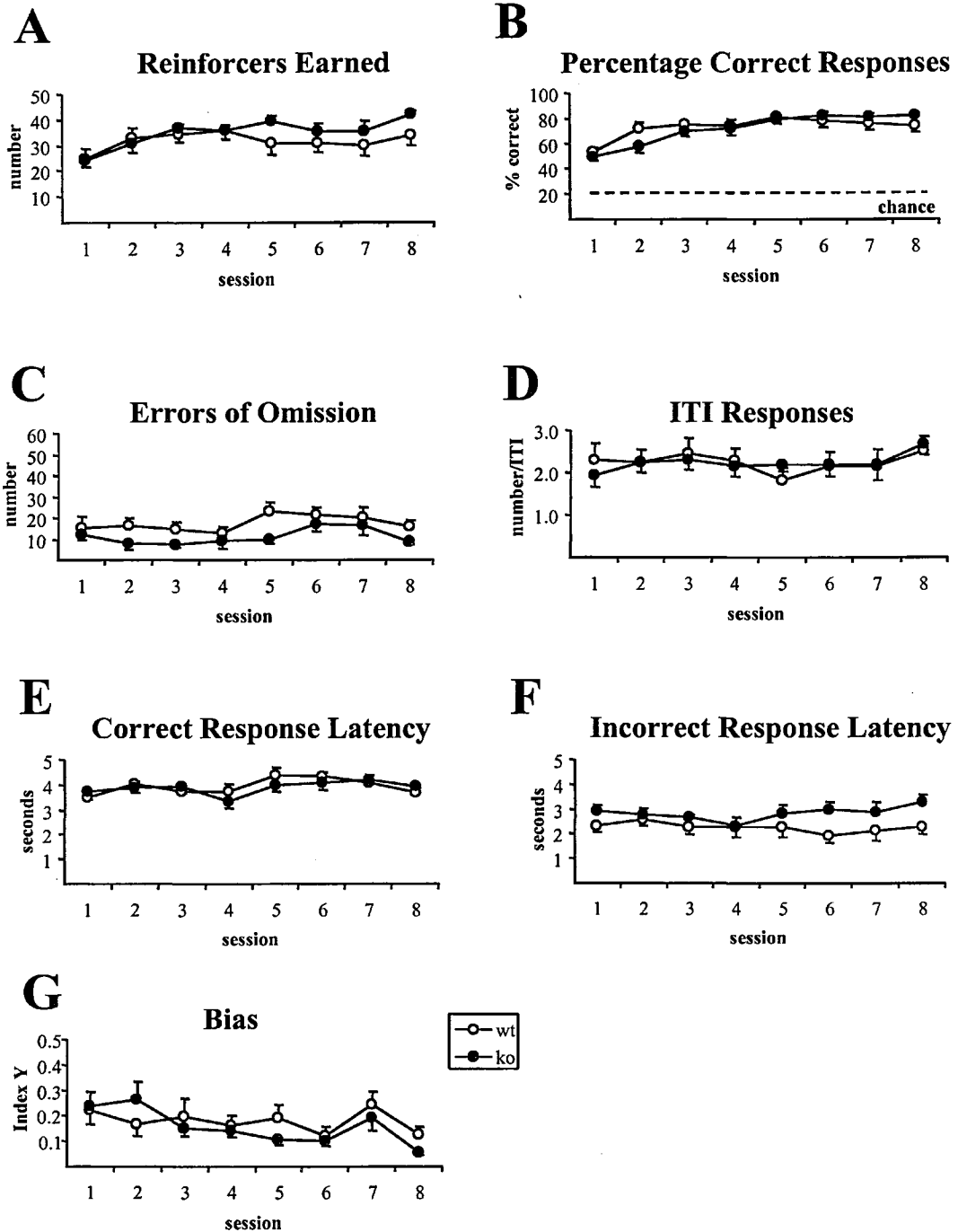


Figure 10.6 Five-choice simultaneous discrimination in mice lacking CRHR1. Animals were tested for 8 successive sessions, with a stimulus duration of 8 sec: wildtype mice (wt), CRHR1^{-/-} mutant (ko). (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding, (data are expressed as means and SEMs).

Except for the response latencies, there was no effect of genotype on any measure during this stage (number of reinforcers earned genotype effect $F_{1,18} = 0.006$; genotype \times stimulus duration effect $F_{3,54} = 1.34$; genotype \times session effect $F_{4,72} = 0.32$; genotype \times stimulus duration \times session effect $F_{12,216} = 0.42$; percentage correct responses: genotype effect $F_{1,18} = 0.13$; genotype \times stimulus duration effect $F_{3,54} = 2.01$; genotype \times session effect $F_{4,72} = 0.41$; genotype \times stimulus duration \times session effect $F_{12,216} = 0.50$; errors of omission: genotype effect $F_{1,18} = 0.06$; genotype \times stimulus duration effect $F_{3,54} = 0.64$; genotype \times session effect $F_{4,72} = 0.52$; genotype \times stimulus duration \times session effect $F_{12,216} = 0.56$; ITI responses: genotype effect $F_{1,18} = 0.12$; genotype \times stimulus duration effect $F_{3,54} = 0.29$; genotype \times session effect $F_{4,72} = 1.16$; genotype \times stimulus duration \times session effect $F_{12,216} = 0.33$; bias: genotype effect $F_{1,18} = 0.07$; genotype \times stimulus duration effect $F_{3,54} = 2.20$; genotype \times session effect $F_{4,72} = 1.74$; genotype \times stimulus duration \times session effect $F_{12,216} = 0.79$, all p 's > 0.050 ; Figures 10.7A – D, G). Overall, there was a tendency for CRHR1 knockout mice to have longer correct response latencies, which just failed significance (genotype effect $F_{1,18} = 4.08$, $p = 0.059$), but ANOVA did not reveal any significant interaction effect (genotype \times stimulus duration effect $F_{3,54} = 2.12$; genotype \times session effect $F_{4,72} = 1.13$; genotype \times stimulus duration \times session effect $F_{12,216} = 1.19$, all p 's > 0.050 ; Figure 10.7E). Moreover, overall incorrect response latencies were higher in mutants relative to wildtype littermates (genotype effect $F_{1,18} = 5.63$, $p = 0.029$), but again there was no interaction effect (genotype \times stimulus duration effect $F_{3,54} = 0.83$; genotype \times session: $F_{4,72} = 0.96$; genotype \times stimulus duration \times session effect $F_{12,216} = 1.31$, all p 's > 0.050), indicating a parallel shift (Figure 10.7F).

Baseline responding of CRHR1 knockout mice

Subsequently, mice received nine more session with 0.5 s stimulus duration. Over repeated testing with this stimulus duration a mild but significant accuracy difference became apparent in that the relative number of correct responses made by wildtype mice was higher than in mice lacking CRHR1 (Table 10.1). However, when this parameter was analysed over all sessions with 0.5 s stimulus duration (including the first 5 sessions), only a strong tendency remained (genotype effect $F_{1,19} = 4.34$, $p = 0.051$), indicating the weakness of this effect. There was no further difference between groups (Table 10.1).

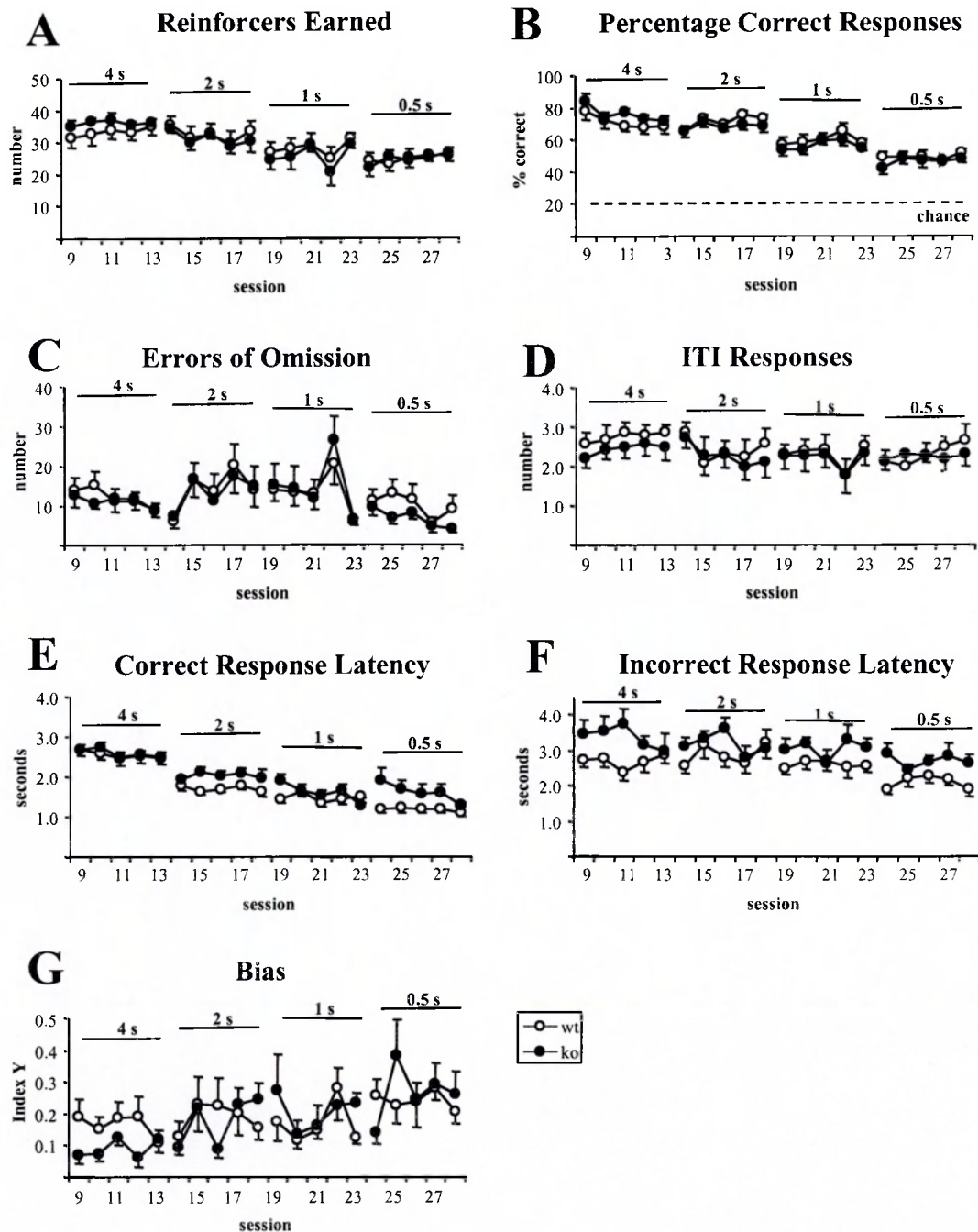


Figure 10.7 Effects of gradual reduction of the stimulus duration on 5-CSRTT performance in mice lacking CRHR1. Animals were tested for 5 sessions for each stimulus duration: wildtype mice (wt), CRHR1^{-/-} mutant (ko). (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding, (data are expressed as means and SEMs).

Table 10.1 Baseline performance of CRHR1 knockout mice (means \pm SEMs, averaged over nine sessions).

Measure	wildtype	knockout	genotype effect (F(1,19))	genotype \times session (F(8,152))
No. Reinforcers Earned	28.64 \pm 1.40	26.40 \pm 1.78	0.99	0.49
% Correct Responses	56.72 \pm 1.69	50.07 \pm 2.16	5.90*	0.84
Errors of Omission	9.39 \pm 1.96	6.96 \pm 2.50	0.45	0.74
No. ITI Responses	2.11 \pm 0.22	1.89 \pm 0.28	0.37	0.20
Corr. Resp. Lat. (sec)	1.17 \pm 0.06	1.19 \pm 0.13	0.02	0.69
Incorr. Resp. Lat. (sec)	2.23 \pm 0.20	2.47 \pm 0.16	1.45	1.15
Bias (Index Y)	0.18 \pm 0.02	0.23 \pm 0.02	3.05 ⁺	0.56

*P = 0.025; ⁺P = 0.097; only P-values < 0.1 are indicated; No.: number; Corr. Resp. Lat.: correct response latency; Incorr. Resp. Lat.: incorrect response latency.

Effects of decreasing the ITI duration

Shortening of the ITI led to a decrease in the number of reinforcers earned (ITI reduction effect $F_{2,38} = 20.41$, $p < 0.001$; Figure 10.8A), in percentage correct responding (ITI reduction effect $F_{2,38} = 4.31$, $p = 0.039$; Figure 10.8B), increased errors of omission (ITI reduction effect $F_{2,38} = 5.13$, $p = 0.012$; Figure 10.8C), correct (ITI reduction effect $F_{2,38} = 11.84$, $p < 0.001$; Figure 10.8E) and incorrect (ITI reduction effect $F_{2,38} = 21.68$, $p < 0.001$; Figure 10.8F) response latencies, but had no significant effect on biased responding (ITI reduction effect $F_{2,38} = 0.34$, $p > 0.05$) (Figures 10.8A -C, D, F). An overall effect of genotype was seen in the number of reinforcers earned (genotype effect $F_{1,19} = 6.60$, $p = 0.019$) and in percentage correct responses (genotype effect $F_{1,19} = 5.35$, $p = 0.032$) during this stage of the experiment, while no further effect of genotype or genotype \times ITI reduction interaction was found (reinforcers earned: genotype \times ITI reduction effect $F_{2,38} =$

0.14; percentage correct responses: genotype \times ITI reduction effect $F_{2,38} = 0.08$; errors of omission: genotype effect $F_{1,19} = 0.31$; genotype \times ITI reduction effect $F_{2,38} = 0.27$; correct response latency: genotype effect $F_{1,19} = 0.33$; genotype \times ITI reduction effect $F_{2,38} = 1.66$; incorrect response latency: genotype effect $F_{1,19} = 0.37$; genotype \times ITI reduction effect $F_{2,38} = 1.59$; bias: genotype effect $F_{1,19} = 2.89$; genotype \times ITI reduction effect $F_{2,38} = 0.14$; all p 's > 0.050).

Effects of increasing the ITI duration

Conversely, when the length of the ITI was gradually increased from 5 to 20 s, only tendencies were seen for the number of reinforcers earned (ITI increment effect $F_{2,38} = 3.37$, $p = 0.062$) and for percentage correct responding (ITI increment effect $F_{2,38} = 3.37$, $p = 0.064$) to change with the duration of the ITI, while no effect of ITI duration was observed on any of the other measures (Figures 10.9A – F). There was no group difference in terms of accurate responding when ITI duration was increased (genotype effect $F_{2,38} = 0.38$; genotype \times ITI increment effect $F_{2,38} = 0.38$, both p 's > 0.050 ; Figure 10.9B), but genotypes differed again in incorrect response latencies (genotype effect $F_{2,38} = 5.52$, $p = 0.03$; genotype \times ITI increment effect $F_{2,38} = 1.47$, $p > 0.05$), with mutant mice performing at higher latencies (Figure 10.9F). There was also a tendency for CRHR1 knockout mice to make more biased responses (genotype effect $F_{2,38} = 3.22$, $p = 0.088$; genotype \times ITI increment effect $F_{2,38} = 0.08$, $p > 0.05$; Fig. 9D), but no other effect was seen (number of reinforcers earned: genotype effect

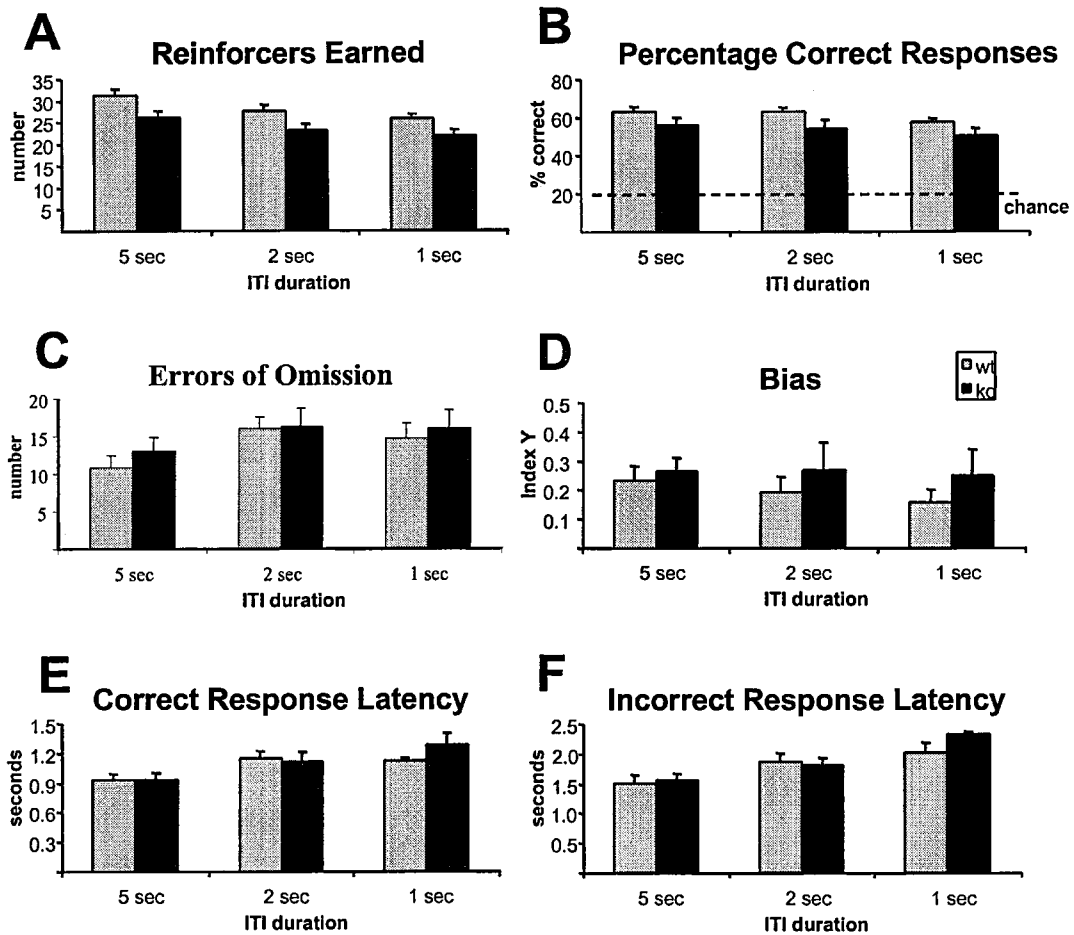


Figure 10.8 Effects of decreasing the ITI duration in CRHR1 knock out animals. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) biased responding, (E) correct response latency, (F) incorrect response latency, (data are expressed as means and SEMs).

$F_{2,38} = 0.86$, genotype \times ITI increment effect $F_{2,38} = 0.19$; errors of omission: genotype effect $F_{2,38} = 0.73$, genotype \times ITI increment effect $F_{2,38} = 0.37$; correct response latency: genotype effect $F_{2,38} = 0.96$, genotype \times ITI increment effect $F_{2,38} = 0.004$, all p 's > 0.050). Thus, decreasing the predictability of stimulus onset by increasing the ITI had no effect on accurate responding.

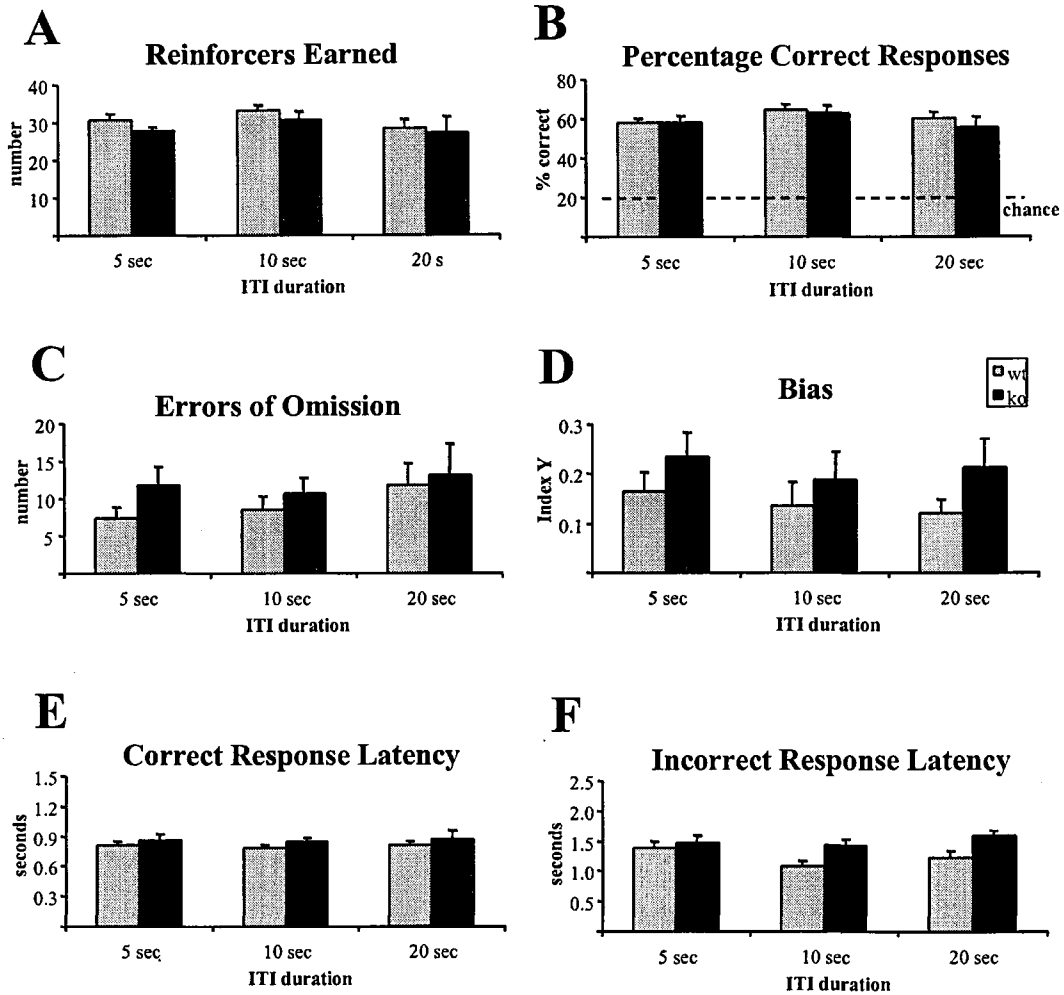


Figure 10.9 Effects of increasing the ITI duration in mice lacking CRHR1. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) biased responding, (E) correct response latency, (F) incorrect response latency, (data are expressed as means and SEMs).

Discussion

Accurate performance as well as response latencies in this task depended on stimulus duration and declined with shorter stimuli. Likewise, a reduction in predictability of stimulus onset (high rate of stimulus presentation, earlier than expected) led to a

reduction in accurate responding. Both correct and incorrect response latencies increased as a function of reduced stimulus predictability. Interestingly, increasing the ITI duration, i.e., a low rate of stimulus presentation, failed to significantly affect these measures, suggesting that once animals were expecting the stimulus they were attentive and waiting for the stimulus to be presented. In addition, accurate performance on this task was sensitive to the disrupting effects of scopolamine, comparable to those found in experiments presented in Chapters 8 and 9. White noise, on the other hand, left accuracy relatively unaffected (cf. percentage correct responses in the dose-response curve in Figure 10.1B with percentage correct responses in the presence of white noise in Figure 10.2B), which is also in line with earlier findings on the effects of white noise on accurate performance in mice tested in a five-choice serial reaction time (Chapter 9; Humby et al., 1999).

Acute administration of the selective CRHR1 antagonist R121919 dose-dependently increased errors of omission and a concomitant tendency for a dose-dependent decrease in the number of reinforcers earned was observed under basal conditions. Notably, neither percentage correct responses nor correct response latencies were affected by the drug – measures which both have been suggested to be predictive for disrupted attention in this task (Blondel et al., 2000; Carli et al., 1983). This suggests that acute blockade of CRHR1 has no effect on target scanning, but exerts non-specific effects on responsivity. This notion receives support by the data obtained during presentation of white noise. Again, the high dose of R121919 impaired errors of omission and also the number of reinforcers earned, but had no effect on the relative number of correct responses or correct response latency.

The effects of combined treatment with scopolamine and R121919 were mainly independent. As before, R121919, at a dose of 40 mg/kg, increased errors of omission and decreased the number of reinforcers earned, but had no effect on accuracy, while scopolamine at the 0.5 mg/kg dose impaired accuracy and also decreased the number of reinforcers earned. Both drugs together had additive effects on the latter measure, but drugs failed to potentiate each other. Another interaction between scopolamine and R121919 was observed in terms of an increase in correct response latencies, while the CRHR1 antagonist failed to alter the effects of scopolamine on percentage correct responding. Thus, there is, albeit limited, interaction between acute CRHR1 blockade and the cholinergic system.

Mice lacking CRHR1 did not differ from controls during autoshaping or during acquisition of the discrimination rule. The only difference during the gradual reduction of stimulus duration was an increased response latency in CRHR1 knockout mice. However, during sessions with shortest stimulus duration (0.5 s), a small group difference in accurate responding developed. This difference, albeit significant, was marginal (56.72 % vs. 50.07 %). The fact that groups only started to dissociate after a few sessions argues against a pure attentional deficit and opens the possibility that other factors contributed to these results.

This accuracy deficit was also seen under conditions of short ITI durations, i.e., when stimuli were presented at faster rate than expected, but it was independent on the length of the ITI, which would also argue against a pure attentional deficit. An increase of ITI duration seemed to have even beneficial effects in that groups did no

longer differ during these latter sessions with the exception of an increase in incorrect response latencies in mutant mice.

Chapter 11

The role of CRHR2 on five-choice serial reaction time performance in mice

Introduction

Overstimulation of CRH receptors result in an impairment in visuospatial attentional processes assessed in 5-CSRTT in mice overexpressing CRH. However, lack of CRH does not affect these processes. Acute blockade of CRHR1 subtype exerts non-specific effects on responsivity in this task, whereas life-long lack of CRHR1 subtype results in mild impairments in 5-CSRTT performance, which is likely not a pure attentional deficit. Here, the functional role of the CRHR2 subtype in visuospatial attention was investigated.

Material and Methods

Animals

Male CRHR2 deficient mice were provided by M. Stenzel-Poore (10 wildtype, 12 heterozygote, 12 knockout). Mutants were generated as described previously by Coste and colleagues (Coste et al., 2000). In brief, this CRHR2-deficient mouse line was established by deleting the coding sequences of the transmembrane regions III and IV of the CRHR2 gene. This resulted in a truncated protein unable to activate the adenylate cyclase second messenger system or to transmit any ligand-induced signals. Animals were housed individually and maintained on a 12:12 h light/dark cycle (lights on at 06:00 hours) with *ad lib* water throughout. They were maintained at 85-90% of their free feeding weight, starting one week prior to the beginning of the experiment by restricting the laboratory chow access to 3.0-3.5 g / day / mouse (depending on the number of pellets eaten during testing). All experiments were conducted during the light phase of the cycle.

5-CSRT

Five sessions were scheduled per week (one session per day, Monday-Friday). Animals were trained as described in detail in Chapter 2. After habituation, animals were shaped using the autoshaping procedure. Seven sessions were scheduled during this experimental stage. Then, animals were trained to learn visual discrimination. After stable performance was reached, the stimulus duration was gradually reduced (i.e., 8 s, 4 s, 2 s, 1 s, 0.5 s), with 5 sessions per stimulus duration.

Manipulations of basic task parameters

After animals reached stable performance, a series of manipulations of the basic task were made which were designed to increase the attentional load. First, the effects of the presence of a distractor (a white noise stimulus (1.2 kHz, 76 dB), presented during the 500 ms preceding the stimulus onset), were studied. Second, the ITI durations were gradually reduced (5.0, 2.5, 1.0 seconds) after which they were increased (5.0, 7.5, 10 seconds). Third, the stimulus duration was gradually decreased (0.5, 0.3, 0.2, 0.1 sec).

Cued detection

The predictability of the stimulus was enhanced by introduction of a pre-sound (1.2 kHz, 76 dB), presented during the 500 ms preceding light stimulus onset (cued detection). Five sessions were scheduled using this procedure.

Effects of pharmacological treatment on baseline performance

Five sessions were scheduled where animals received i.p. saline injections prior to test. R121919 was dissolved in vehicle (3 % Tween 20 in saline) and injected i.p. in a volume of 10 ml/kg, 20 min before testing (40 mg/kg). Scopolamine hydrochloride (Sigma Chemicals, Deisenhofen, Germany) was dissolved in saline and injected i.p. in a volume of 10 ml/kg, 20 min before testing (0.0, 0.02, 0.10, 0.5 mg/kg). Treatment was given in ascending order, and at least two days lapsed in-between drug sessions.

Results

Autoshaping procedure

All groups completed nearly all 20 trials within the time limits of 20 min from the first session onwards. No significant effects of genotype or session were found (genotype effect $F_{2,3} = 0.63$; session effect $F_{6,180} = 1.28$; genotype x session effect $F_{12,180} = 0.80$, all p 's > 0.050) (Figure 11.1A). The number of ITI responses decreased over sessions ($F_{6,180} = 9.54$, $p < 0.001$), however no effect of genotype was found (genotype effect $F_{2,3} = 1.41$; genotype x session effect $F_{12,180} = 1.13$, both p 's > 0.050) (Figure 11.1B). Likewise, the time to complete a trial decreased over sessions ($F_{6,180} = 15.96$, $p < 0.001$) (Figure 11.1C). There was a tendency for a gene-dose effect for this parameter, i.e. the overall time per trial showed a rank order (knockout $>$ heterozygote $>$ wildtype) which, however, just failed significance ($F_{2,3} = 2.68$, $p = 0.085$). No genotype x session interaction was found for this parameter ($F_{12,180} = 1.64$, $p > 0.05$). Furthermore, no differences of genotype or session were found in terms of trials completed prior to pellet delivery (genotype effect $F_{2,3} = 0.51$; session effect $F_{6,180} = 2.12$; genotype x session effect $F_{12,180} = 1.15$, all p 's > 0.050) (Figure 11.1D).

Simple visual discrimination learning

Both groups of animals responded above chance from the first session onwards. All groups increased performance over sessions in terms of percentage correct responding ($F_{10,310} = 38.78$, $p < 0.001$), although overall performance was dependent on genotype ($F_{2,31} = 4.93$ $p = 0.014$), whereas no genotype x session interaction was found ($F_{20,310} = 1.38$, $p > 0.05$) (Figure 11.2B).

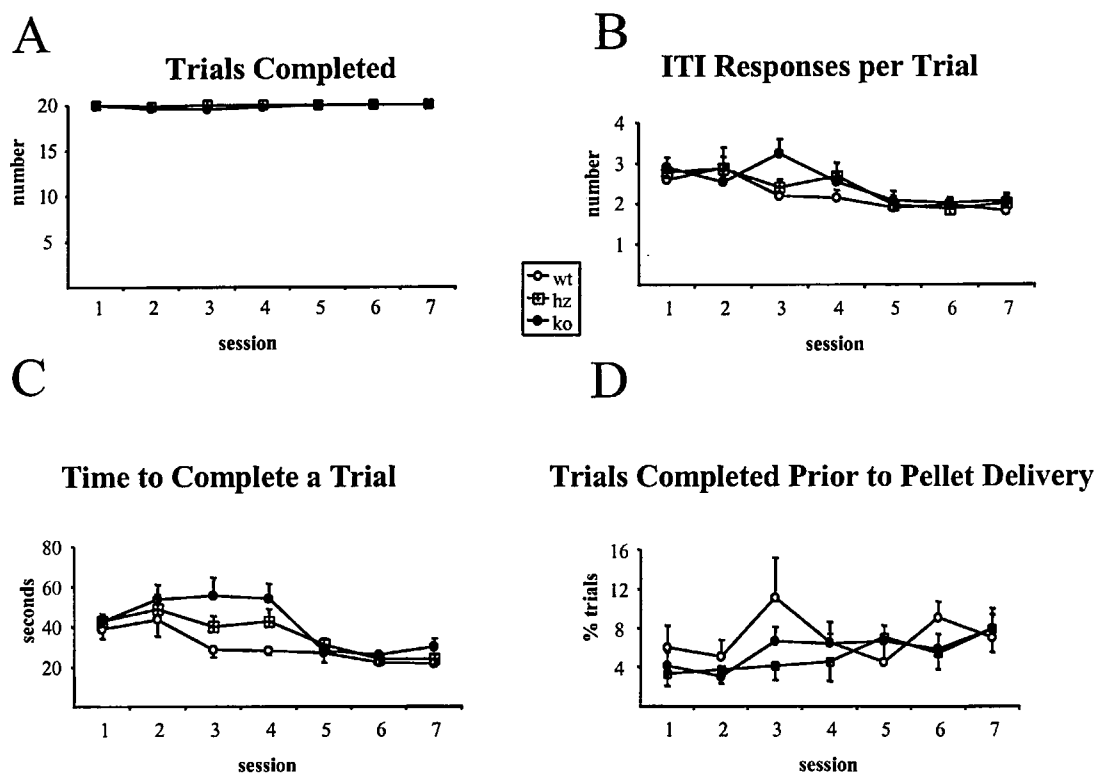


Figure 11.1 Effects of (partial) lack of CRHR2 on autoshaping performance. Animal were tested for 7 successive sessions. (wt) wildtype mice, (hz) heterozygote mice, (ko) knockout mice. (A) Number of trials completed, (B) ITI responses per trial, (C) time to complete a trial, (D) average response latency and (E) trials completed prior to pellet delivery. Data are presented as means, with error bars denoting SEMs.

Post hoc testing revealed that knock mice showed an decreased percentage correct responding compared heterozygote animals ($p < 0.05$), however, compared to wildtype animals it just failed significance ($p = 0.084$). No difference was found between heterozygotes and wildtypes. Likewise, reinforcers earned increased over sessions session ($F_{10,310} = 22.64$, $p < 0.001$), whereas no effect of genotype was found (genotype effect $F_{2,31} = 0.64$; genotype x session effect $F_{20,310} = 1.58$, both p 's > 0.050) (Figure 11.2A). A concomitant decrease in errors of omission occurred over sessions ($F_{10,310} = 2.77$, $p = 0.032$), while no effect of genotype was found (genotype effect $F_{2,31} = 0.00$; genotype x session effect $F_{20,310} = 2.02$, both p 's > 0.050) (Figure

11.2C). Furthermore, correct response latency decreased over sessions ($F_{10,300} = 3.62$, $p < 0.010$), but incorrect response latency did not ($F_{10,300} = 1.15$, $p > 0.05$) (Figures 11.2E, F). No effects of genotype was found in terms of correct (genotype effect $F_{2,30} = 0.55$; genotype x session effect $F_{20,300} = 0.93$, both p 's > 0.050), and incorrect (genotype effect $F_{2,30} = 0.78$; genotype x session effect $F_{20,300} = 0.68$, both p 's > 0.050) response latency. An increase in ITI responses ($F_{10,310} = 4.74$, $p < 0.001$) and a decrease in biased responding ($F_{10,310} = 7.08$, $p < 0.001$) was seen over sessions (Figures 11.2D, G). For both parameters, the genotype effect failed to reach significance (ITI responses: genotype effect $F_{2,31} = 0.05$; genotype x session effect $F_{20,310} = 1.38$; biased responding: genotype effect $F_{2,30} = 1.91$; genotype x session effect $F_{20,300} = 1.16$, all p 's > 0.050).

Reduction of stimulus duration

Decreasing the stimulus duration led to a decrease in percentage correct responses ($F_{3,93} = 170.52$, $p < 0.001$) and decreased the number of reinforcers ($F_{3,93} = 30.05$, $p < 0.001$) (Figures 11.3A, B). Despite the increasing attentional load, errors of omission also declined with decreasing stimulus duration ($F_{3,93} = 17.03$, $p < 0.001$) (Figure 11.3C). Furthermore, both correct and incorrect latencies decreased with decreasing stimulus duration ($F_{3,93} = 17.24$, $p < 0.001$ and $F_{3,90} = 32.82$, $p < 0.001$, respectively) (Figures 11.3E, F). Likewise, the number of responses made during the ITI decreased with shorter stimulus duration (effect $F_{3,93} = 16.38$, $p < 0.001$), whereas biased responding increased as a consequence of decreasing stimulus duration ($F_{3,93} = 26.66$, $p < 0.001$) (Figure 11.3D, G).

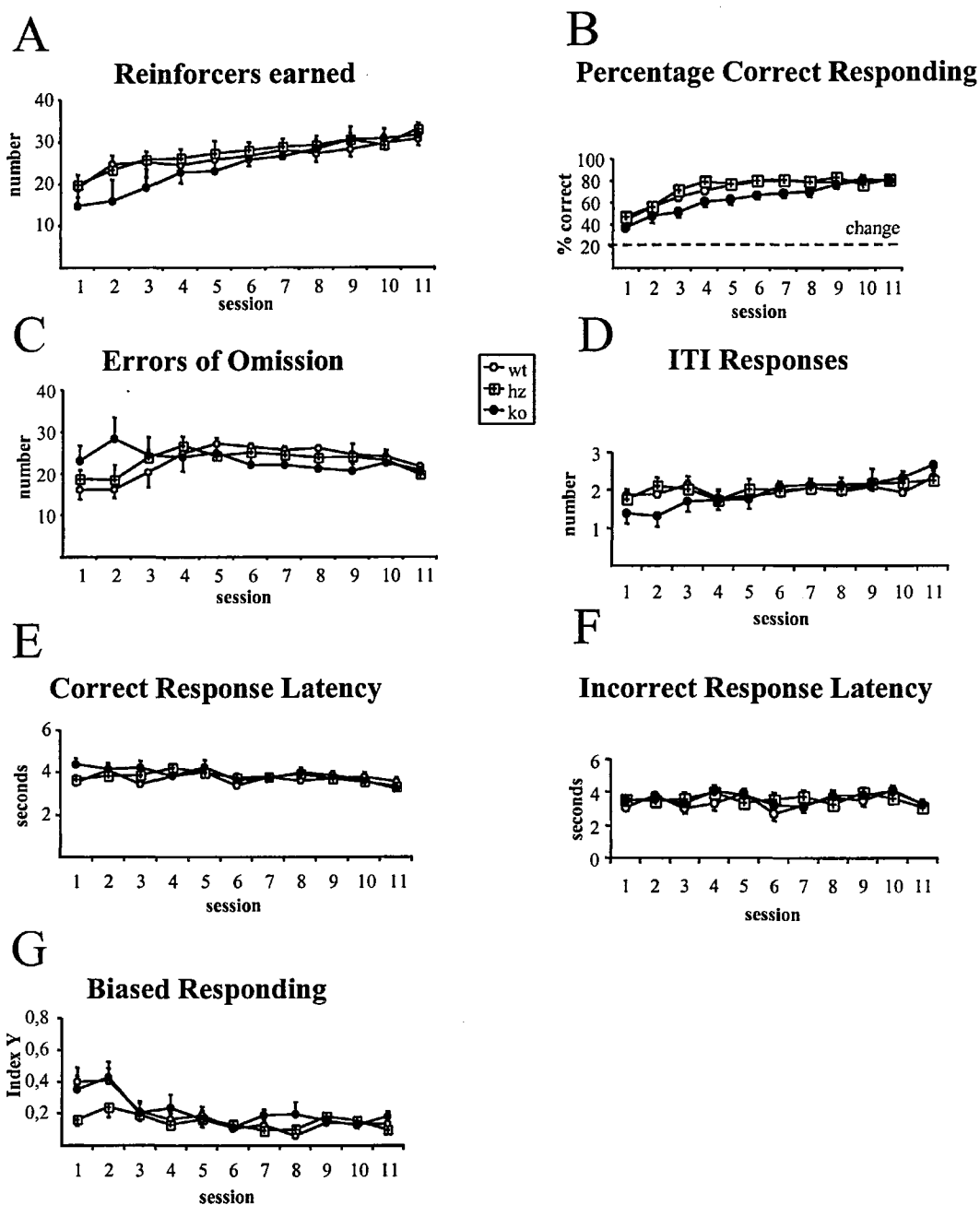


Figure 11.2 Effects of (partial) lack of CRHR2 on five-choice simultaneous discrimination performance. Animals were tested for 11 successive sessions, with a stimulus duration of 8 sec. (wt) wildtype mice, (hz) heterozygote mice, (ko) knockout mice. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding (index Y). Data are presented as means, with error bars denoting SEMs

No significant differences between groups were found during this testing phase: accurate responding (genotype effect $F_{2,31} = 0.53$; genotype \times stimulus duration interaction effect $F_{6,93} = 0.47$; genotype \times stimulus duration \times session interaction effect $F_{24,372} = 1.23$), reinforcers earned (genotype effect $F_{2,31} = 1.26$; genotype \times stimulus duration interaction effect $F_{6,93} = 0.19$; genotype \times stimulus duration \times session interaction effect $F_{24,372} = 0.87$), errors of omission made (genotype effect $F_{2,31} = 1.68$; genotype \times stimulus duration interaction effect $F_{6,93} = 0.45$; genotype \times stimulus duration \times session interaction effect $F_{24,372} = 1.36$), number of ITI responses (genotype effect $F_{1,11} = 2.12$; genotype \times stimulus duration interaction effect $F_{3,33} = 0.12$; genotype \times stimulus duration \times session interaction effect $F_{12,132} = 0.24$), correct response latency (genotype effect $F_{2,31} = 0.91$; genotype \times stimulus duration interaction effect $F_{6,93} = 1.22$; genotype \times stimulus duration \times session interaction effect $F_{24,372} = 0.50$, all p 's > 0.05), incorrect response latency (genotype effect $F_{2,30} = 0.81$; genotype \times stimulus duration interaction effect $F_{6,90} = 0.84$; genotype \times stimulus duration \times session interaction effect $F_{24,360} = 1.07$) and biased responding (genotype effect $F_{2,31} = 1.38$; genotype \times stimulus duration interaction effect $F_{6,93} = 0.60$; genotype \times stimulus duration \times session interaction effect $F_{24,372} = 0.99$) (Figure 11.3, all p 's > 0.05).

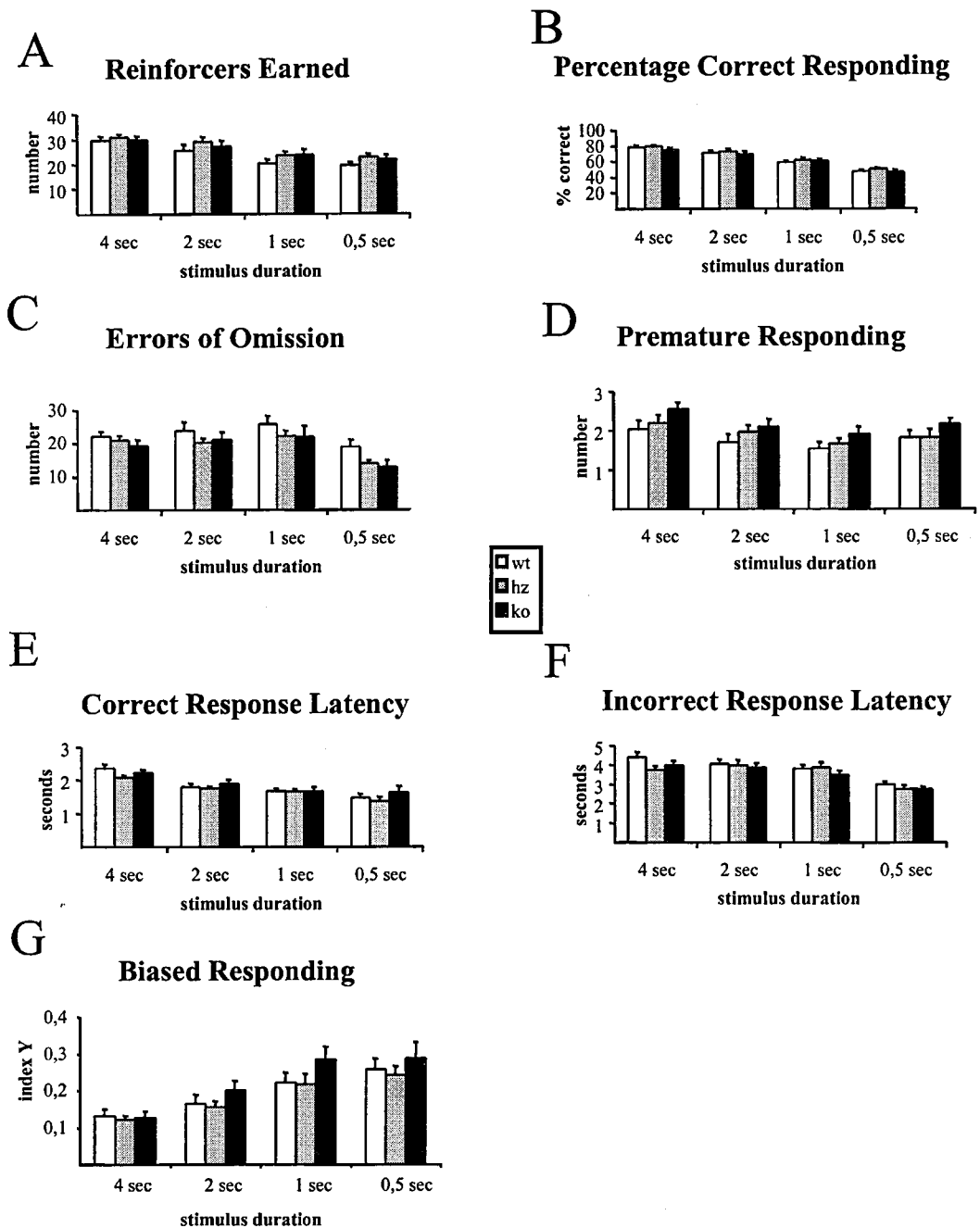


Figure 11.3 Effects of gradual reduction of the stimulus duration on 5-CSRTT performance in mice (partially) lacking CRHR2. Animals were tested for 5 sessions for each stimulus duration. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

Baseline performance

Subsequently, mice received fifteen more sessions with a stimulus duration of 0.5 second. Over repeated testing with this stimulus duration (twenty sessions, including the first 5 sessions), no significant differences were found in the performance of knockout mice compared to wildtype littermates (Table 11.1), except for the errors of omission (genotype effect $F_{2,31} = 3.54$, $p = 0.041$). Post hoc testing revealed that knockout mice made less errors of omission compared to wildtype littermates ($p < 0.050$).

Table 11.1 Baseline performance in CRHR2 knockout mice (means \pm SEMs, averaged over twenty sessions).

Measure	wildtype	heterozygote	knockout	genotype effect [$F_{(2,31)}$]	genotype \times session [$F_{(26,403)}$]
No. Reinforcers Earned	20.96 \pm 1.19	24.62 \pm 1.13	24.00 \pm 1.36	2.34	1.04
% Correct Responses	48.26 \pm 1.74	52.29 \pm 2.55	49.07 \pm 2.66	0.78	0.81
Errors of Omission	16.19 \pm 1.78	12.43 \pm 1.15	12.43 \pm 1.15*	3.54	0.45
No. ITI Responses	1.81 \pm 0.17	1.71 \pm 0.16	2.07 \pm 0.12	1.62	0.50
Corr. Resp. Lat. (sec)	1.43 \pm 0.07	1.25 \pm 0.09	1.41 \pm 0.13	0.98	0.59
Incorr. Resp. Lat. (sec)	2.85 \pm 0.16	2.66 \pm 0.11	2.70 \pm 0.14	0.51	0.93
Bias (Index Y)	0.26 \pm 0.02	0.24 \pm 0.02	0.22 \pm 0.03	0.67	1.27

All p 's > 0.050 , except for errors of omission ($p = 0.041$); No.: number; Corr. Resp. Lat.: correct response latency; Incorr. Resp. Lat.: incorrect response latency, * $p < 0.050$ compared to wildtype

Noise disturbance

Presenting white noise prior to a session, led to an increase in the number of errors of omission ($F_{1,31} = 8.07$, $p = 0.008$) and the responses made during the ITI ($F_{1,31} = 14.31$, $p < 0.001$) (Figures 11.4C, D). No further effects of white noise were seen: number of reinforcers earned ($F_{1,31} = 0.96$), percentage correct responding ($F_{1,31} = 0.80$, $p > 0.05$) (Figures 11.4A, B), correct response latency ($F_{1,31} = 0.16$), incorrect latency $F_{1,31} = 0.96$) and biased responding ($F_{1,31} = 2.02$) (all p 's > 0.05).

The errors of omission tended to be lower in mutants during this experimental phase, but this effect, failed to reach significance (genotype effect $F_{2,31} = 3.06$, $p = 0.062$). Genotype and noise presentation did not interaction ($F_{1,31} = 0.18$, $p > 0.05$). No further differences of genotype were found in this experimental phase: percentage correct responding (genotype effect $F_{2,31} = 0.17$, genotype x noise effect $F_{1,31} = 2.37$), reinforcers earned (genotype effect $F_{2,31} = 0.83$, genotype x noise effect $F_{1,31} = 2.74$), correct response latency (genotype effect $F_{2,31} = 0.22$, genotype x noise effect $F_{2,31} = 0.12$), incorrect response latency (genotype effect $F_{2,31} = 0.61$, genotype x noise effect $F_{2,31} = 0.55$), ITI respondings (genotype effect $F_{2,31} = 0.47$, genotype x noise effect $F_{2,31} = 0.55$) and biased responding (genotype effect $F_{2,31} = 0.33$, genotype x noise effect $F_{2,31} = 0.74$) (all p 's > 0.050).

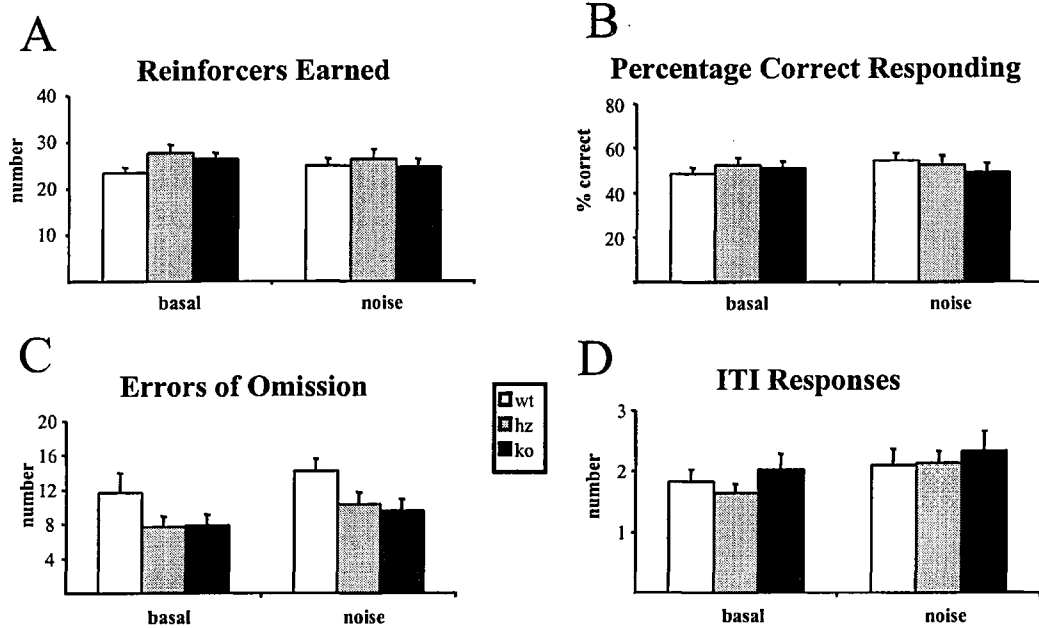


Figure 11.4 Effects of white noise stimulus presented during the 500 ms preceding the stimulus onset on 5-CSRTT performance in mice (partially) lacking CRHR2. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, (F) incorrect response latency, and (G) biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

Effects of decreasing the ITI duration

Reducing the ITI duration resulted a reduction in number of reinforcers earned ($F_{2,62} = 4.71$, $p = 0.017$), together with a concomitant increase in errors of omission (effect $F_{2,62} = 13.94$, $p < 0.001$) (Figures 11.5A, C). No effect on percentage correct responding was seen ($F_{2,62} = 0.35$, $p > 0.05$) (Figure 11.5B). Furthermore, both correct ($F_{2,62} = 12.34$) and incorrect ($F_{2,62} = 10.95$,) response latencies were increased by reducing the ITI duration (both p 's < 0.001 , Figures 11.5E, F). Overall, biased responding was not affected during this experimental stage ($F_{2,62} = 1.70$, $p > 0.05$) (Figure 11.5D).

Although overall biased responding was not significantly different between genotypes (genotype effect $F_{2,31} = 1.87$, $p > 0.05$), a genotype x ITI reduction interaction was found (genotype x ITI reduction $F_{4,62} = 3.86$, $p = 0.006$). Post hoc testing revealed that mice lacking CRHR2 showed an increased biased responding compared to wildtype littermates when the animals were tested with an ITI of 2.5 seconds. No differences depending on genotype were found in this experimental phase: terms of percentage correct responding (genotype effect $F_{2,31} = 2.48$, genotype x ITI reduction $F_{4,62} = 0.86$), reinforcers earned (genotype effect $F_{2,31} = 0.48$, genotype x ITI reduction $F_{4,62} = 0.45$, both), errors of omission (genotype effect $F_{2,31} = 1.98$, genotype x ITI reduction $F_{4,62} = 0.72$), correct response latency (genotype effect $F_{2,31} = 2.48$, genotype x ITI reduction $F_{4,62} = 0.86$) and incorrect response latency (genotype effect $F_{2,31} = 2.48$, genotype x ITI reduction $F_{4,62} = 0.86$) (all p 's > 0.050).

Effects of increasing the ITI duration

Reducing the ITI duration led to an increase in percentage correct responding ($F_{2,62} = 4.99$, $p = 0.013$) and in the number of reinforcers earned ($F_{2,62} = 12.88$, $p < 0.001$) (Figures 11.6A, B). Furthermore, the errors of omission were decreased ($F_{2,62} = 5.46$, $p = 0.013$), as well as incorrect response latency ($F_{2,62} = 6.47$, $p = 0.005$), whereas only a tendency in reduction of correct response latency was seen ($F_{2,62} = 2.98$, $p = 0.072$) (Figures 11.6C, E, F). Increasing the ITI duration did not affect biased responding (ITI increment effect $F_{2,62} = 0.60$, $p > 0.05$) (Figures 11.6D).

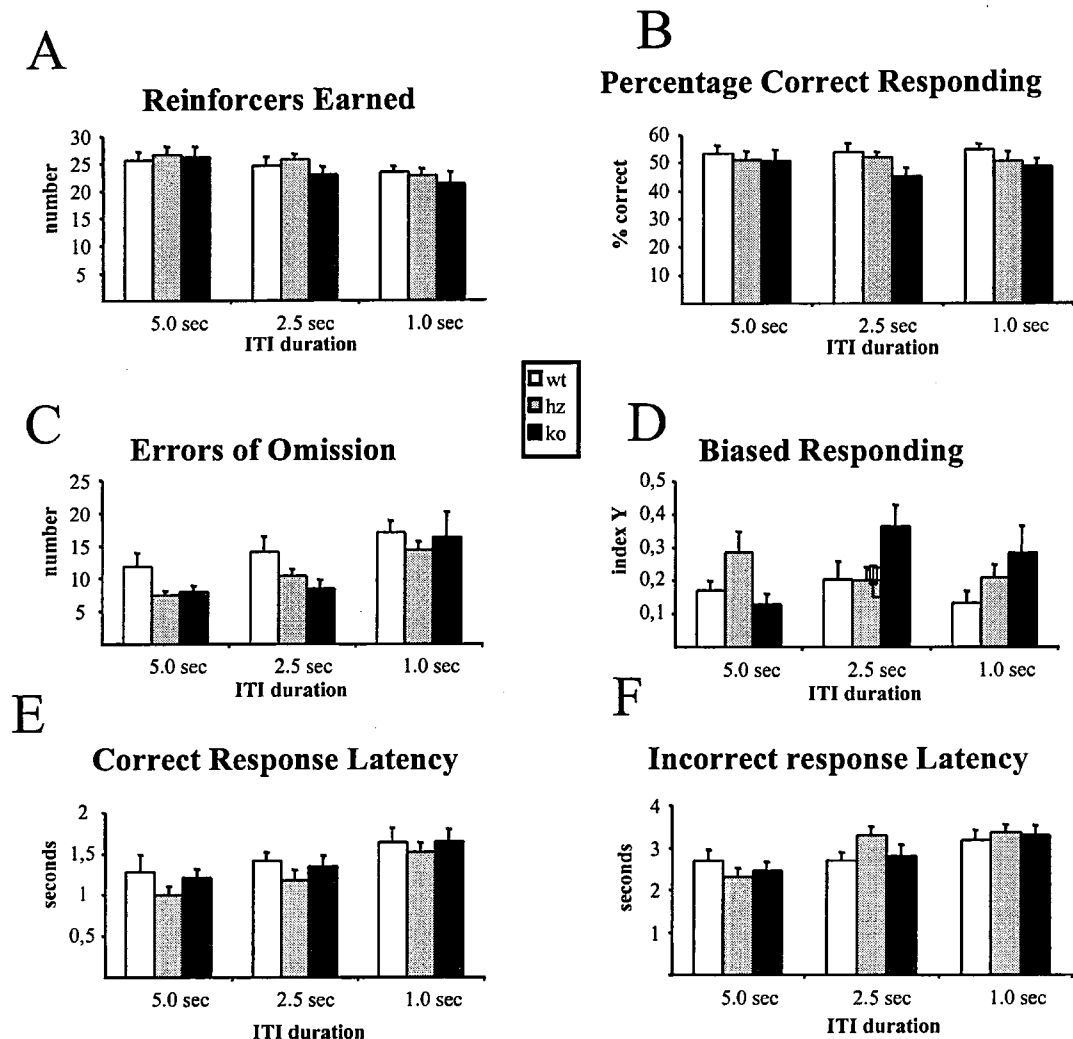


Figure 11.5 Effects of reduction of ITI duration on 5-CSRTT performance in mice (partially) lacking CRHR2. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) biased responding (index Y), (E) correct response latency, and (F) incorrect response latency. Data are presented as means, with error bars denoting SEMs.

No effect of genotype was found during this experimental phase: percentage correct responding (genotype effect $F_{2,31} = 0.27$, genotype x ITI reduction $F_{4,62} = 2.09$), reinforcers earned (genotype effect $F_{2,31} = 0.17$, genotype x ITI reduction $F_{4,62} = 2.25$, both), errors of omission (genotype effect $F_{2,31} = 2.00$, genotype x ITI reduction $F_{4,62} = 0.49$) (all p 's > 0.050), correct response latency (genotype effect $F_{2,31} = 0.15$, genotype x ITI reduction $F_{4,62} = 0.13$), incorrect response latency (genotype effect $F_{2,31} = 2.34$, genotype x ITI reduction $F_{4,62} = 0.21$) and biased responding (genotype effect $F_{2,31} = 0.20$, genotype x ITI reduction $F_{4,62} = 0.14$) (all p 's > 0.050).

Effects of further reduction of stimulus duration

Decreasing the stimulus duration reduced accurate responding ($F_{3,93} = 24.30$, $p < 0.001$) and reinforcers earned ($F_{3,93} = 32.95$, $p < 0.001$), while errors of omission remained unaffected ($F_{3,93} = 0.81$, $p > 0.05$) (Figures 11.7A - C). Furthermore, both correct ($F_{3,93} = 3.26$, $p = 0.035$) and incorrect ($F_{3,93} = 4.64$, $p = 0.009$) response latencies were increased as a consequence of reducing the stimulus duration (Figures 11.7E, F). ITI responses were also reduced ($F_{3,93} = 6.48$, $p < 0.002$), whereas no effect on biased responding was seen ($F_{3,93} = 0.72$, $p > 0.05$) (Figure 11.7D).

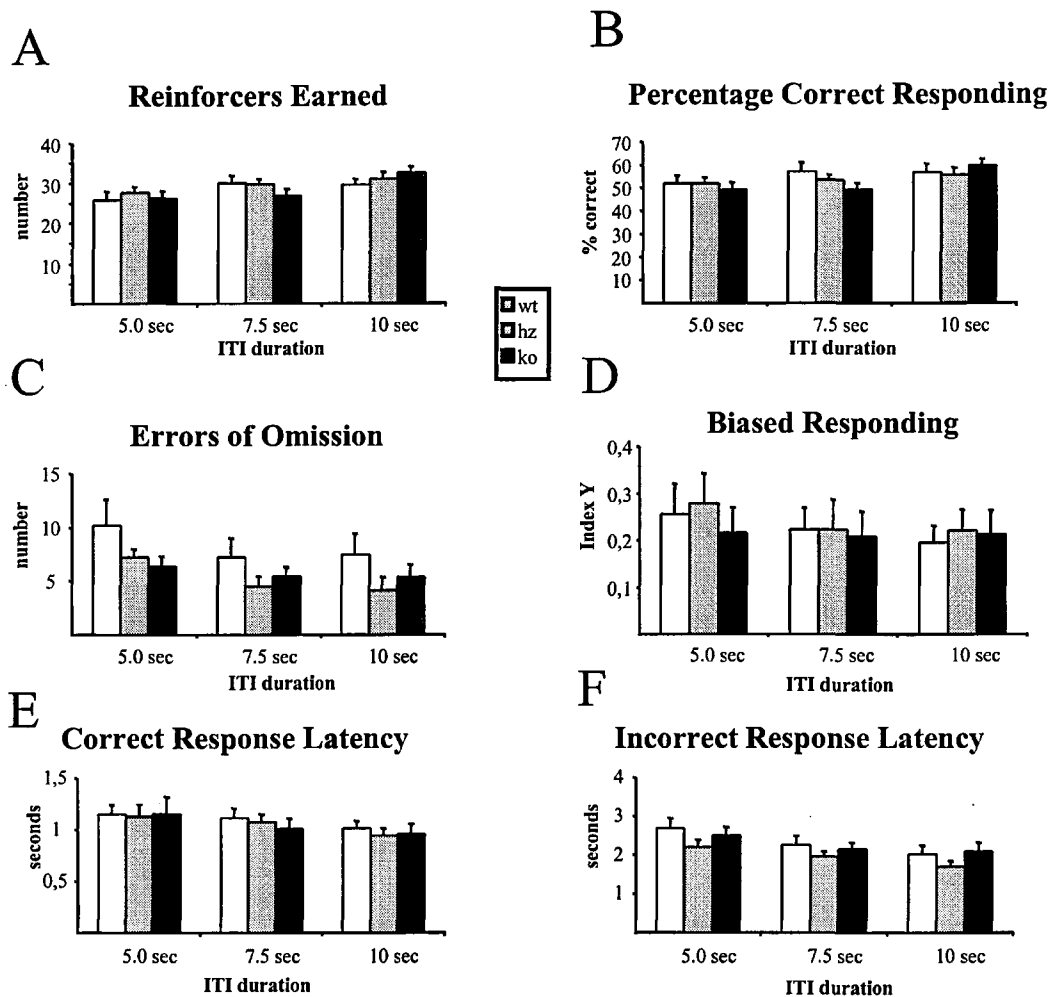


Figure 11.6 Effects of increasing the ITI duration in mice (partially) lacking CRHR2. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) biased responding (index Y), (E) correct response latency, and (F) incorrect response latency. Data are presented as means, with error bars denoting SEMs.

Groups did not differ in percentage correct responding (genotype effect $F_{1,11} = 0.37$, genotype x stimulus duration reduction $F_{6,93} = 1.01$, both p 's > 0.050) or number of reinforcers earned (genotype effect $F_{1,11} = 0.37$, genotype x stimulus duration reduction $F_{6,93} = 1.01$, both p 's > 0.050). Furthermore, no interaction was seen between genotype and reduction of stimulus duration (genotype x stimulus duration reduction $F_{6,93} = 1.67$, $p > 0.05$). However, heterozygote and knockout animals made less errors of omission than wildtype littermates (genotype effect $F_{1,11} = 8.32$, $p < 0.001$).

Cued detection

Mice were tested under condition of cued detection for 5 sessions. No group differences were found (Table 11.2).

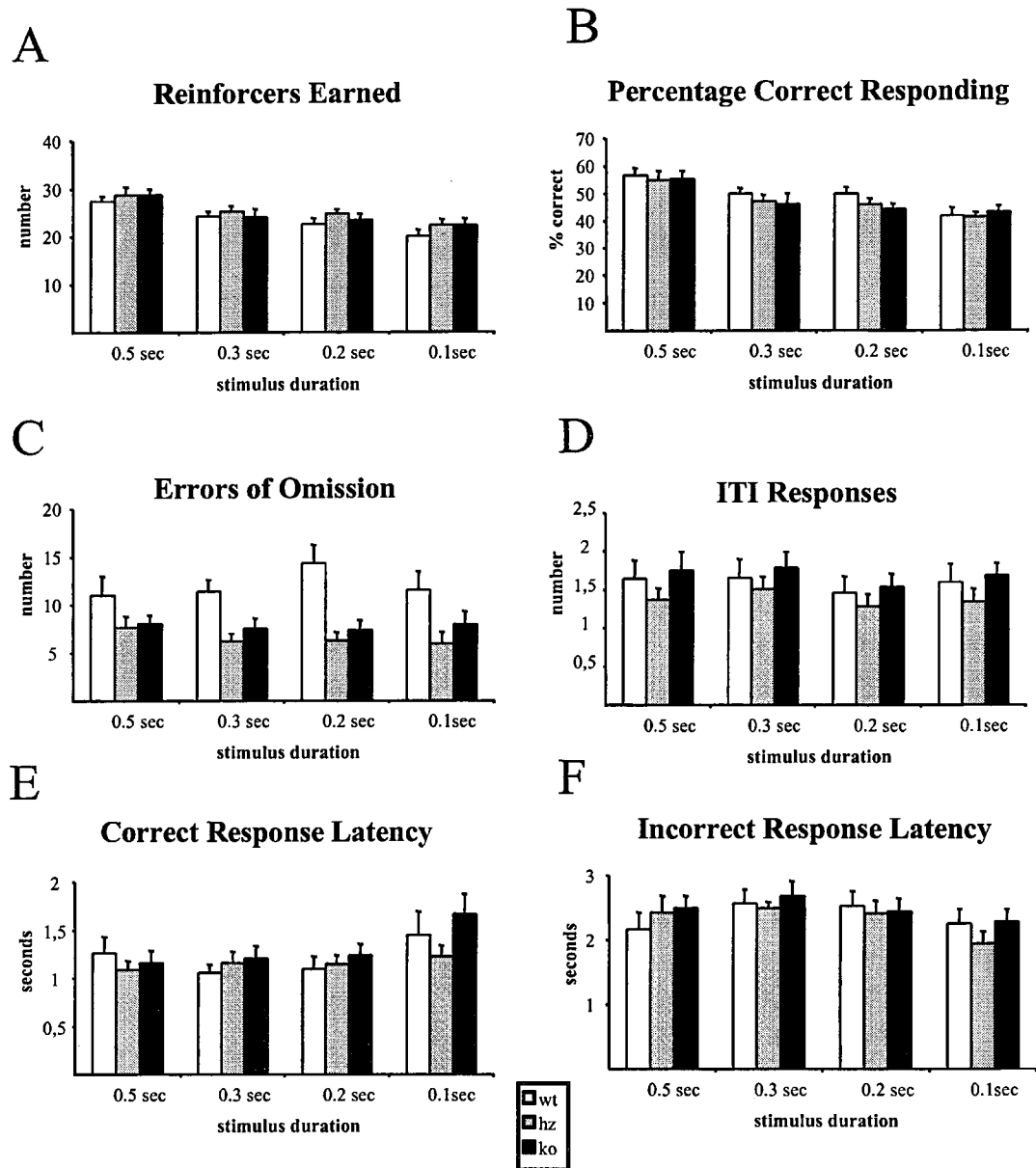


Figure 11.7 Effects of reduction of stimulus duration on 5-CSRTT performance in mice (partially) lacking CRHR2. (A) Number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) ITI responses per trial, (E) correct response latency, and (F) incorrect response latency. Data are presented as means, with error bars denoting SEMs.

Table 11.2 Cued detection in CRHR2 knockout mice (means \pm SEMs, averaged over five sessions).

Measure	wildtype	heterozygote	knockout	genotype effect F values [F _{2,31}]
No. Reinforcers Earned	23.16 \pm 1.02	24.00 \pm 0.88	23.65 \pm 1.30	0.14
% Correct Responses	47.92 \pm 3.19	45.35 \pm 1.52	44.97 \pm 2.69	0.39
Errors of Omission	10.36 \pm 2.00	6.83 \pm 1.41	6.78 \pm 1.57	1.46
No. ITI Responses	1.53 \pm 0.21	1.43 \pm 0.12	1.57 \pm 0.13	0.26
Corr. Resp. Lat. (sec)	1.23 \pm 0.14	0.96 \pm 0.10	1.00 \pm 0.11	0.67
Incorr. Resp. Lat. (sec)	1.96 \pm 0.25	1.70 \pm 0.25	1.54 \pm 0.26	0.66
Bias (Index Y)	0.29 \pm 0.05	0.28 \pm 0.05	0.27 \pm 0.03	0.08

All p 's > 0.050 ; No.: number; Corr. Resp. Lat.: correct response latency; Incorr.

Resp. Lat.: incorrect response latency

Effects of acute scopolamine treatment

Percentage correct responding was not affected during this phase of testing (treatment effect $F_{3,81} = 1.43$, genotype effect $F_{2,27} = 0.58$, treatment \times genotype effect $F_{6,81} = 0.44$, all p 's > 0.050) (Figure 11.8B). However, scopolamine reduced the number of reinforcers earned ($F_{3,81} = 29.26$, $p < 0.001$) while no effects of genotype were found (genotype effect $F_{2,27} = 2.38$, treatment \times genotype effect $F_{6,81} = 0.32$, both p 's > 0.050) (Figure 11.8A). Scopolamine treatment resulted in an concomitant increase in errors of omission ($F_{3,81} = 61.81$, $p < 0.001$; treatment \times genotype effect $F_{6,81} = 0.27$, $p > 0.050$) (Figure 11.8C). No differences in correct response latency were found (treatment effect $F_{3,81} = 0.77$, genotype effect $F_{2,27} = 1.56$, treatment \times genotype effect $F_{6,81} = 0.20$, all p 's > 0.050), and no genotype or treatment effects were found in term of incorrect response latency (treatment effect $F_{3,81} = 1.67$, genotype effect $F_{2,27} = 0.47$, both p 's > 0.050). A significant genotype \times treatment interaction was found for the latter parameter ($F_{6,81} = 2.84$, $p = 0.020$) (Figures 11.8E, F), but post hoc analysis failed to reveal a difference of individual

dosages. In addition, ITI responses decreased by scopolamine treatment ($F_{3,78} = 12.74$, $p < 0.001$) while biased responding increased by treatment ($F_{3,81} = 61.81$, $p = 0.003$) (Figures 11.8D, G). No effects of genotype were found for either parameters (ITI responses: genotype effect $F_{2,26} = 0.94$, treatment x genotype effect $F_{6,78} = 1.03$; biased responding: genotype effect $F_{2,26} = 1.05$, treatment x genotype effect $F_{6,78} = 1.23$, all p 's > 0.050).

Effects of acute R121919 treatment

Percentage correct responding remained unaffected by treatment ($F_{1,27} = 0.00$, $p > 0.05$) (Figure 11.9B). However, the number of reinforcers earned decreased as a function of dose ($F_{1,27} = 25.97$, $p < 0.001$), whereas errors of omission concomitantly increased ($F_{1,27} = 27.77$, $p < 0.001$) (Figure 11.9A, C). Furthermore, both correct ($F_{1,27} = 7.54$, $p = 0.011$) and incorrect ($F_{1,27} = 4.70$, $p = 0.039$) response latencies were increased after treatment (Figures 11.9E, F). In addition, ITI respondings was decreased by treatment ($F_{1,27} = 5.88$, $p = 0.023$), while biased responding remained unaffected ($F_{1,26} = 1.03$, $p > 0.05$) (Figures 11.9D, G). No effect of genotype were found in terms of percentage correct responding (genotype effect $F_{2,26} = 0.37$, genotype x treatment effect $F_{2,27} = 1.41$), reinforcers earned (genotype effect $F_{2,26} = 2.58$, genotype x treatment effect $F_{2,27} = 0.02$), errors of omission (genotype effect $F_{2,26} = 1.66$, genotype x treatment effect $F_{2,27} = 0.46$), ITI responses (genotype effect $F_{2,26} = 1.11$, genotype x treatment effect $F_{2,27} = 0.58$), biased responding (genotype effect $F_{2,26} = 0.54$, genotype x treatment effect $F_{2,26} = 0.12$) and correct response latency (genotype effect $F_{2,26} = 2.69$, genotype x treatment effect $F_{2,27} = 2.10$) (all p 's > 0.050). Group differences just failed significance for incorrect response latency ($F_{2,26} = 3.34$, $p = 0.051$), and a significant interaction between genotype and

treatment was found (genotype x treatment effect $F_{2,27} = 4.55$, $p = 0.020$). Post hoc testing revealed that knockout mice showed an increase in incorrect response latency compared to wildtype ($p < 0.010$) and heterozygote ($p < 0.050$) mice.

Discussion

All groups performed equally well during the autoshaping procedure. Animals performed above chance level during simple visual discrimination learning, indicating they all learned the association between the stimulus and reward. Although all groups improved in performance over sessions in terms of accurate responding, knockout animals showed impaired performance. However, during the phase reduction of stimulus duration, homozygote knockout animals performed as accurate as the other groups. This suggests that mice lacking the CRHR2 can learn to visual discriminate as well as wildtype littermate, but learn at a lower rate.

The data presented in this and in previous chapters show that increasing the attentional load by reducing the stimulus duration result in a decrease in number of reinforcers earned, in percentage correct responding, response latencies and ITI responding, independent of genotype. Strikingly, the errors of omission also decreased following reduction of the stimulus duration. Furthermore, both correct and incorrect latencies decreased and dissociated: under long stimulus duration, correct and incorrect response latencies did not differ, whereas corrects responses were made faster than incorrect responses with shortening of stimulus duration, when performance started to be attentionally demanding.

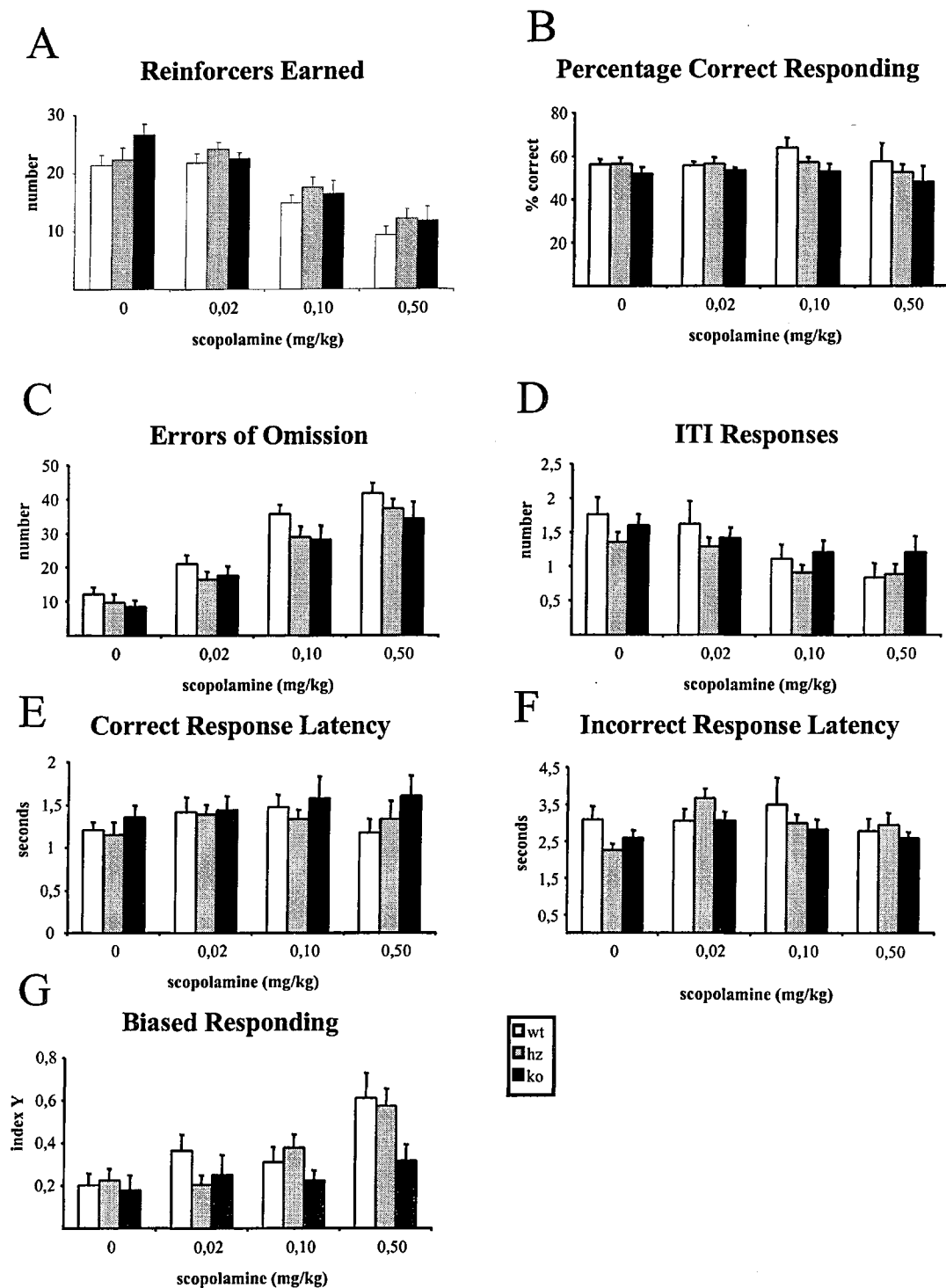


Figure 11.8 Effects of scopolamine on 5-CSRTT in mice (partially) lacking CRHR2. Scopolamine was administered at dosages of 0.0, 0.02, 0.1, 0.5 and 1.0 mg/kg i.p., 20 min prior to testing. Treatment was given in ascending order. (A) number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) incorrect response latency, (E) ITI responses per trial (F) Biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

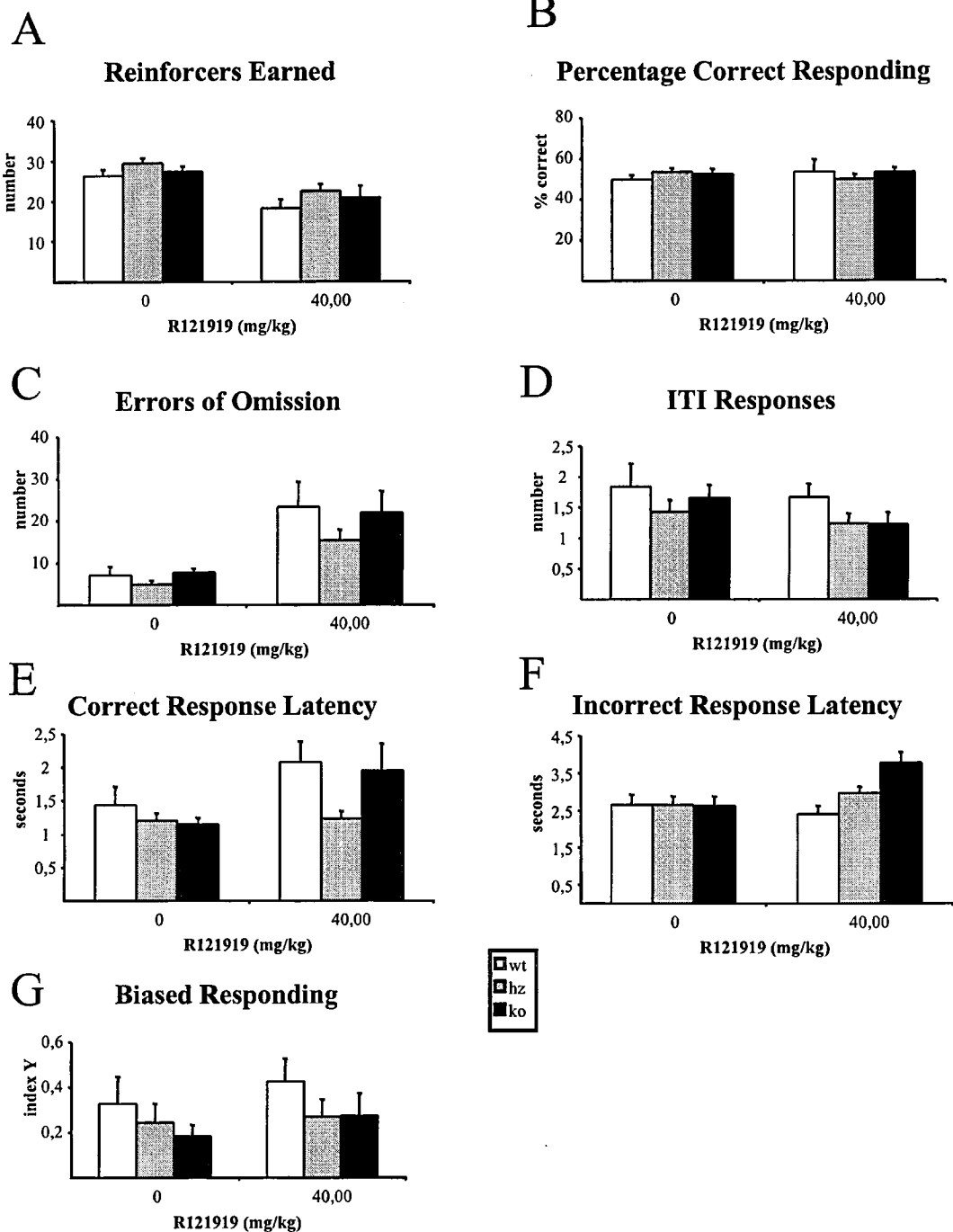


Figure 11.9 Effects of R121919 (40 mg/kg) on 5-CSRTT in mice (partially) lacking CRHR2. (A) number of reinforcers earned, (B) percentage correct responding, (C) number of errors of omission, (D) incorrect response latency, (E) ITI responses per trial (F) Biased responding (index Y). Data are presented as means, with error bars denoting SEMs.

Extended testing with 0.5 sec stimulus duration revealed that knockout animals made less errors of omission, while no other parameter differed during this experimental stage between groups, suggesting that lack of CRHR2 has no effect on target scanning, but exerts non-specific effects on responsivity. Furthermore, groups failed to differ when predictability of the stimulus was enhanced by cued detection.

After baseline performance was stable, test conditions were changed in order to increase task difficulty. Thus, these data as well as from previous studies demonstrate that presentation of a pre-sound before stimulus presentation had no effect on performance, suggesting that mice do not benefit from this manipulation (cued detection, Bushnell, 1995).

A range of alterations of basic parameters was applied in order to increase the attentional load. These alteration changed performance of mice in distinct ways: white noise primarily increased errors of omission. Although a previous experiment revealed an increase in accuracy (Chapter 9), such effect was absent in the present study. Reducing the ITI duration resulted in a reduction in reinforcers earned, an increase of errors of omission and an increase in correct response latencies. A reduction in accurate responding was observed in the study presented in Chapter 10 but not in the present study. Furthermore, no significant effects of ITI reduction on performance were observed in the study presented in Chapter 9. These differences between these studies might be due to the small groups size in this study, and indicates the subtlety of the altered performance. Increasing the ITI duration did not alter performance in two studies, but increased accurate responding, reinforcers earned, and both latencies in another. This suggests that the attentional load increases following reduction of the ITI, but not due to unpredictability of the stimulus.

Reducing the stimulus duration below 0.5 sec resulted in reduced accurate responding, but left errors of omission unaffected. Reinforcers earned were concomitantly decreased. Furthermore, correct and incorrect latencies were decreased.

Taken together, an increase in attentional load can be achieved by reducing the ITI duration or by reducing the stimulus duration, which is reflected by the effects on percentage correct responding and correct response latencies, measures which both have been suggested to be predictive for attention in this task (Blondel et al., 2000; Carli et al., 1983). An increase in ITI duration only appeared to decrease attentional load, whereas presentation of white noise prior to stimulus duration resulted in non-specific effects on responsivity.

In the present study, alterations of basic parameters did not induce group differences, except that knockout mice showed an increased bias during an ITI duration of 2.5 sec only. Furthermore, knockout animals showed a reduced rate of errors of omission when the stimulus duration was reduced. Under baseline conditions it reached significance only when compared over several sessions, indicating the weakness of the effect. Attentional performance remained also stable in mutants when the attentional load was increased, or when the predictability of the stimulus was reduced or in presence of a disrupting stimulus.

Blockage of muscarinic receptors by scopolamine treatment resulted in a reduction of reinforcers earned and an increment of errors of omission. Strikingly, and in contrast to the effect of scopolamine seen in other studies (Chapter 8, 9 and 10), no effect on

accurate responding was found. Furthermore, it is noteworthy that the disruptive effects of scopolamine occurred at lower dosages compared to other studies (Chapter 8, 9 and 10). This could be due to difference in genetic background of the animals used in the different experiments. Alternatively, it is possible that the animals used different response strategies in the different experiments. Scopolamine treatment resulted in an increase in bias. This indicates that animal focus more on one side of the box. Such performance results in an easier strategy, but can be without effect on accurate responding (neglecting one side will increase the errors of omission, leaving accuracy unaffected). These scopolamine-induced changes occurred independent of genotype, suggesting that loss of CRHR2 does not alter the disrupting effect of scopolamine.

Treatment with R121919 resulted in an increase in errors of omission, a decrease in reinforcers earned, leaving percentage correct responding unaffected. Similar effects were reported in Chapter 8 and 10, albeit, less intense. Furthermore, R121919 treatment also increased response latencies, whilst ITI responses were reduced. Thus, comparable to scopolamine treatment, R121919 treatment induced stronger disrupting effects with the applied dosage, which could be due to differences in genetic background.

Blocking CRHR1 in CRHR2 knockout animals should result in total blockade of CRH activity. Despite this, no effect were found on attention, which suggests that inactivation of the CRH system does not impair attentional processes, as measured in the 5-CSRTT, which is in line with the results obtained with CRH knockout mice (Chapter 9). However, overproduction of CRH can impair attention. Although these

disruptive effects could not be blocked acutely with R121919, long term administration may improve attention or blockade of CRHR2 may be beneficial, at least in improving attentional processes, in diseases in which attention is impaired and a hyperactive CRH system is involved such as depression.

Chapter 12

Discussion

The focus of the present work was to investigate the role of the CRH system in emotional function and attention. In the first part of the work, anxiety-related behaviour was investigated. Since there is evidence from the literature that different anxiety tasks may measure different aspects of anxiety, a battery of anxiety tests was employed. Indeed, this was found in several experiments presented in this thesis, emphasising the importance to use multiple test for assessment of anxiety-related behaviour. This is, at least in part, due to the fact that: “all models used to date have limitations and no single paradigm adequately models all aspects of anxiety” (Weiss et al., 2000).

This test battery employed was also used to investigate the effects of life-long exposure to excessive CRH on anxiety-related behaviour. CRH overexpressing mice displayed increased anxiety-related behaviour in the light dark box, and a similar tendency was found in the open field and in time spent grooming after arousal, which was comparable to the anxiogenic effects of acute central CRH administration (Dunn and Berridge, 1990). In striking contrast, conflict behaviour and conditioned fear,

tested 24 h after conditioning, were not affected by CRH overproduction. Even a reduced emotional response was found in conditioned fear when tested 1 hour after conditioning. The lack of increase in conditioned fear might be due to an impairment in learning and/or memory (see discussion Chapter 5). However, this would not explain the lack of overproduction of CRH on conflict behaviour. Alternatively, it is possible that CRH affects only a subset of the different types of anxiety measured in these paradigms. Thus, live-long overproduction of CRH may increase some, but not all aspects of anxiety.

The role of CRH on different aspects of Anxiety

A comparable distinction in the role of CRH in fear and anxiety has been proposed by Michael Davis and co-workers, based on studies of the startle reflex in rats. The startle reflex in rodents can be elicited by a loud sound. Presentation of a cue prior to the sound which was previously associated with an aversive stimulus (e.g. an footshock), potentiates the startle reflex (Brown et al., 1951). Such phenomenon is known as fear-potentiated startle. The startle response can also be potentiated by i.c.v. administration of CRH, and this effect lasts for several hours. This is independent of HPA-axis activity, as adrenalectomy and lesioning the PVN has no effect on CRH-induced potentiation (Lee et al. 1994; Liang et al., 1992). Both forms of potentiation can be reduced by several classes of clinical active anxiolytics (Swerdlow et al., 1986; Lee et al., 1994). However, CRH potentiated startle is, in contrast to fear-potentiated startle, a form of long-lasting, unconditioned anxiety. Fear potentiated startle, on the other hand, is believed to be an index of fear.

A distinct functional anatomy underlies these forms of potentiation. Lesioning the basolateral or central nucleus of the amygdala blocks fear potentiated startle (Campeau and Davis, 1995; Hitchcock and Davis, 1986), while lesions of the BNST leave fear potentiated startle unaffected (Hitchcock and Davis, 1991). In striking contrast, lesioning the central nucleus of the amygdala fails to block CRH-potentiated startle, whereas lesioning the BNST blocks the effects of CRH completely (Lee and Davis, 1997). Furthermore, the enhancement of startle by i.c.v. administration of CRH can be mimicked by microinfusion of CRH directly into the BNST (Lee and Davis, 1997). In addition, i.c.v. CRH-enhanced startle can be reduced by local administration of a CRH antagonist into the BNST.

Based on the above described evidence, it has been suggested that the BNST may be a system that responds to signals more akin to anxiety than fear, whereas the central nucleus of the amygdala is clearly involved in fear and perhaps less so in anxiety (Lang et al., 2000). Both receive highly processed sensory information from the basolateral nucleus of the amygdala and hence are in a position to respond to emotionally significant stimuli. Furthermore, it was postulated that phasic activation of the amygdala by certain stressors could lead to long-term activation of the BNST via CRH (Lang et al., 2000). This in turn suggest that overproduction of CRH may enhance anxiety, but not fear, which would explain the increased anxiety-related behaviour seen in innate test of anxiety, but not in conditioned fear. This is in line with evidence showing that BNST lesions fail to affect conditioned fear (LeDoux et al. 1988).

Conflict behaviour cannot be classified according to this anxiety / fear distinction and may possibly be an index of yet another aspect of anxiety-related behaviour. Of note, the unaltered conflict behaviour displayed by CRH overexpressing mice contrasts the anxiogenic effects seen after acute centrally administered CRH in conflict tasks (Britton et al., 1985, 2000). In addition, acute administration of CRHR 1 antagonist increased responding in the Vogel conflict test (Millan et al., 2001). This suggests acute challenge of the CRH system can affect conflict behaviour, whereas chronic hyperactivation does not alter conflict behaviour which may reflect compensatory mechanisms, in contrast to the increased anxiety-related behaviour in innate test of anxiety, for example in the light dark exploration task.

Secondary effects of overproduction of CRH

These differences between acute and chronic exposure to CRH could be due to interactions with other neurotransmitter systems. Indeed, data presented in the previous chapters provide evidence that CRH overexpressing mice have altered sensitivity to pharmacological challenges with compounds of several pharmacological classes such as GABA-ergic, serotonergic and cholinergic drugs, which may be absent after acute challenge.

For example, in Chapter 5, it was demonstrated that CRH overexpressing mice are less susceptible to the sedative effects of diazepam. GABA-ergic neurotransmission is potentiated by diazepam via binding to the GABA_A receptors, which results in, among other phenomena, sedation and reduced anxiety-related behaviour. Therefore, it could be speculated that alterations in the GABA-ergic system might be involved in the altered anxiety profile of CRH overexpressors. However, the sedative effects

and anxiolytic effects are mediated by distinct GABA_A receptor subtypes in distinct neuronal circuits (Rudolph and Crestani et al., 1999; McKernan and Rosahl et al., 2000). Therefore, additional experiments assessing GABA-ergic function in CRH overproducing mice in terms of anxiety-related behaviour are necessary to draw firm conclusions.

Perhaps a more likely neurotransmitter system being involved in the altered anxiety-related behaviour of CRH overproducing mice is the serotonergic system. The data presented in Chapter 6 indicate that HPA-axis activation via 5-HT_{1A} receptors is desensitised in CRH overexpressors, whereas hypothermia via 5-HT_{1A} receptors stimulation is unaltered. This suggests that postsynaptic 5-HT_{1A} receptors, probably at the level of PVN, are downregulated, whereas 5-HT autoreceptors remain unaltered (see Chapter 5 for discussion). The importance for alterations in serotonergic neurotransmission secondary to changes in CRH activity for behavioural performance is corroborated by findings that citalopram decreased anxiety-related behaviour in CRH overexpressing animals, while an opposite effect was found in wildtype mice. Surprisingly, the lack of HPA-axis activation after 5-HT_{1A} challenge still emerged in CRH overexpressors. Of note, one of the features often observed after chronic treatment with antidepressants or ECT is a desensitisation of the 5-HT_{1A} autoreceptor. This phenomenon seems to have occurred in both wildtype as well as in transgenic mice

Based on these data, it could be hypothesised that the increase in serotonergic function, which is believed to occur after desensitisation of the 5-HT_{1A} autoreceptor, might be anxiogenic in wildtype animals due to disturbing serotonergic

neurotransmission. In transgenic animals, on the other hand, the beneficial effect of treatment may be due to restoring a disturbed serotonergic system, although it should of course be noted that it cannot be excluded that effects of treatment on anxiety-related behaviour might be due to changes secondary to those of the serotonergic system (for more details see discussion Chapter 7).

Effects of long-term treatment with SSRI's differed not only in terms of anxiety-related behaviour between wildtype and CRH overexpressing animals, but effects on body weight also depended on genotype. Clinical studies have indicated that effects of SSRIs treatment on body weight also depend on the neurobiology of the depressive illness (Harvey and Bouwer, 2000). The data presented here suggest that CRH may play a role in such disease-depend effects.

Apart from alterations in the GABA-ergic and serotonergic system in CRH overexpressing mice, results presented in Chapter 8 provide evidence that the cholinergic system is also affected by overproduction of CRH: mice overproducing CRH were less susceptible to the effects of both blockade and indirect stimulation of cholinergic receptors in the 5-CSRTT. In this task, it has been demonstrated that cholinergic dysfunction can produce deficits in visual attention (Muir et al., 1992), and that these processes can also be disrupted by lesioning the basal cholinergic forebrain (Muir et al., 1994) or the prefrontal cortex (Muir et al., 1996). Moreover, it has recently been demonstrated that extracellular acetylcholine concentration is increased in the prefrontal cortex during performance in the 5-CSRTT (Passetti et al., 2000). Therefore, it is hypothesised that the reduced responsiveness of CRH overproducing mice to the disruptive effects of both blockade and indirect

stimulation of cholinergic receptors may be due to downregulation of cholinergic receptors in consequence of CRH-induced increases in cholinergic activity (Day et al., 1998a, b). However, it cannot be excluded that the reduced susceptibility of the CRH overexpressors to these cholinergic drug is due to alteration in pharmacodynamics and/or pharmacokinetics in these mutants.

Such alterations in cholinergic processes may also affect anxiety-related behaviour at the cortical level (Berntson et al., 1998a). For example, benzodiazepine inverse agonists are anxiogenic and enhance basal forebrain-cortical cholinergic activity, whereas agonists have opposite effects (Sarter and Bruno, 1994; Moore et al., 1993). Moreover, the anxiogenic effect of inverse agonist treatment could be blocked by i.c.v. administration of the cholinergic antagonist carbachol and was also no longer present after selective immunotoxic lesions of basal forebrain cholinergic neurons (Berntson et al., 1996). More recently, it was reported that these effects may be mediated at medial prefrontal level (Hart et al., 1999).

Berntson and colleagues (1998b) hypothesised that “the basal forebrain cholinergic projection is primarily involved in aspects of anxiety that arise from or depend on cortical processing of fear and anxiety-associated stimuli and associations”. This in turn suggests that alterations in the basal forebrain cholinergic system would affect anxiety-related behaviour depending on cortical processing.

One other symptom reported to occur in patients suffering from depression is reduced attentional processes. In order to investigate whether overproduction of CRH

also results in altered attentional processes, CRH transgenic mice were tested in 5-CSRTT.

Measuring attention in mice

The 5-CSRTT has analogies with a range of human attentional paradigms (Mirsky and Rosvold, 1960; Sahakian et al., 1993; Wilkinson, 1963), and provides measures of sustained and divided attention in temporal and spatial domains (Bushnell, 1998; Muir et al., 1996; Robbins et al., 1993). The paradigm was adapted by Carli and colleagues (1983) for testing rats. In this work it has been demonstrated that attentional processes could be assessed in mice in an adapted 5-CSRTT set-up (Chapter 9-11), which has also been shown in other laboratories (Humby et al., 1999).

Muscarinic blockade with scopolamine dose-dependently impaired accuracy and responsivity, as indicated by a decrease in percentage correct responses, in the number of reinforcers earned, and an increase in errors of omission, comparable to what has been reported in related paradigms in rats (Jones and Higgins, 1995; Mirza and Stoleran, 2000; Ruotsalainen et al., 2000) and mice (Humby et al., 1999). However, in the study presented in Chapter 11, scopolamine disrupted performance only in terms of reinforcers earned and errors of omission, leaving accurate responding unaffected. Furthermore, the disruption of performance occurred at lower dosages as in the other studies, and might be due to differences in genetic background. This would be in line with a study reported by Humby and co-workers (1999), showing that the disruptive effects of scopolamine on attentional function in mice differ between strains.

The effects of diazepam were small, leaving accuracy unaffected and, if at all, caused release of responding, as evidenced by an increase in the number of reinforcers earned and an effect on errors of omission, which just failed significance. In similar lines, the benzodiazepine chlordiazepoxide did not affect the ability to discriminate in a simple conditional discrimination task in rats (Dudchenko et al., 1994), and although benzodiazepines impair successive visual discrimination, it has been suggested that this may be related to altered responsivity during trials where withholding of responding would have been appropriate (Cole and Michaleski, 1986; McNaughton, 1985). It is obvious, however, that benzodiazepines affect a range of attentional functions as well (e.g., McGaughy et al., 1994). Unfortunately, there is a relative paucity of published work on the effects of systemic GABA-ergic drugs, in particular of benzodiazepines, on 5-CSRTT performance, and only Muir and colleagues (1992) reported impaired performance in rats following administration of the GABA_A agonist muscimol directly into the basal forebrain.

Overproduction of CRH disturbs sustained and divided attentional processes

CRH overexpressing mice showed impaired autoshaping of a nose-poke response to a light stimulus, as indicated by retarded, but not absent, association between the stimulus and reward, and reduced responsivity. Although subsequent simultaneous discrimination training indicated that transgenic animals had learned some association as they performed above chance level from the first session onwards, they clearly performed below wildtype level. However, both groups of animals improved performance over session, indicating the ability to learn the discrimination in transgenic animals, although they continued to respond with lower accuracy. At

least part of this may be due to different baselines carried over from autoshaping. In addition, biased responding was increased during acquisition and subsequent experimental stages, indicating that transgenic animals reverted to a more simple response strategy, that is to respond as to whether a signal had appeared in a sort of go/no-go like manner, rather than where it had appeared (Marston, 1996; Steckler, 2001).

Extended training with long stimulus durations revealed that transgenic animals were able to reach similar accuracy levels as wildtype animals, although responsivity continued to be reduced, as indicated by a reduction in the number of reinforcers earned, increased errors of omission, decreased responsivity during the ITI, and increased response latencies. Once transgenic mice had reached an accuracy level that was comparable to wildtype performance, they were also able to maintain accuracy to similar degree as wildtypes over a wide range of different stimulus durations. This indicates that learning the discrimination was retarded but remembrance of the response rule appeared to be intact once the discrimination was acquired. Along similar lines, Radulovic and colleagues (1999) suggested that CRH may primarily affect consolidation processes. However, acute local injection of CRH in the brain impairs or facilitates learning in the fear conditioning study depending on brain area, while CRH overexpressors were impaired in learning the appetitive discrimination task in the present set of experiments. Moreover, direct comparison of the two studies is hampered as one uses acute localised infusions, while the other tests animals with a life-long elevation of CRH activity and hence a wide range of potential compensatory processes. Alternatively, this might indicate that CRH facilitates association of an emotional response (fear) with an aversive stimulus, but

might impair learning in an appetitive situation. However, the results from the conditioned fear experiment argue against this possibility. In addition, i.c.v. infusions of CRH(6-33), which displaces CRH from its binding protein, have been shown to improve acquisition of an appetitively motivated visual discrimination in aged rats (Heinrichs et al., 1997), which argues against CRH induced impairment in appetitive learning. Yet another possibility would be that the site of action may be of relevance, as CRH binding protein is predominantly expressed at cortical and hippocampal level (Potter et al., 1992). Thus CRH may facilitate learning at these levels but impair learning when infused into other brain areas (Behan et al., 1995). Furthermore, at least part of the effect of CRH on performance in tasks of learning and memory are due to factors other than effects on learning per se. Indeed, the improvement seen following CRH(6-33) in the study by Heinrichs and colleagues (1997) was seen during early but not late acquisition sessions, arguing against a direct effect on learning. Likewise, we observed a parallel shift in acquisition, i.e., the rate of learning was intact in transgenic animals and, for example, altered responsivity could have affected accuracy in transgenic mice.

Another factor that could interfere with learning would be a change in arousal or attentional processes. Overarousal due to excess CRH could explain the results obtained during acquisition.

When animals were tested for an extended period of 17 sessions with shortest stimulus duration (0.5 s), a mild but significant impairment in percentage correct responding re-emerged in transgenic animals. This was accompanied by longer correct response latencies, while incorrect latencies did not differ between groups.

This accuracy deficit was independent on whether or not a pre-sound was present, suggesting that CRH overexpressing mice did not benefit from this manipulation guiding stimulus detection (cued detection; Bushnell, 1995).

CRH overexpressing mice have been reported to express high levels of ACTH and corticosterone (Stenzel-Poore et al., 1992) and to display hypoactivity when exposed to a novel environment (Chapter 5, Stenzel-Poore et al., 1994), which is comparable to the effects of acute i.c.v. CRH administration, which is even more accentuated following social defeat stress (Stenzel-Poore et al., 1994). Furthermore, these animals show enhanced anxiety-related behaviour in several anxiety paradigms. Therefore, it is noteworthy that transgenic animals seemingly showed a behavioural response that was similar to the one seen in wildtype mice in the five choice paradigm. One explanation for this discrepancy could be that animals were highly familiarised with the present task, while exaggerated behavioural responses following CRH administration or in animals overexpressing CRH are primarily seen following exposure to novel situations (Stenzel-Poore et al., 1994). In addition, CRH overexpressors display hypolocomotor activity when tested in range of tasks, which is not longer present after habituation to the test apparatus (Chapter 5). Along similar lines, diazepam failed to affect performance in transgenic animals. Another benzodiazepine with comparable anxiolytic, amnestic and sedative properties, chlordiazepoxide, has been reported to attenuate the performance deficit in CRH overexpressing mice tested in a water maze place navigation task (Heinrichs et al., 1996). But here again, animals are exposed to a highly novel situation. In the familiar operant task, transgenic mice only differed from wildtype mice in a stronger behavioural disinhibition induced by diazepam. This renders explanations that

performance deficits seen in transgenic mice on this task are due to altered anxiety unlikely. Therefore, it is suggested that chronic excessive CRH exposure results in a mild attentional deficit.

Sustained and divided attention remains intact without CRH

Since it is possible that CRH is not only disruptive, but that a central level of CRH activity is necessary for adequate level of arousal and attention, CRH knockout were tested in 5-CSRTT. However, in Chapter 9, it was demonstrated that lack of CRH does not alter the learning of the association of the light with the reinforcement, simultaneous discrimination, or visual spatial attention under basal conditions, under conditions with an increased attentional load, or after stress exposure. This suggests that either CRH has only disruptive properties in attentional processes, but is not required for proper visuospatial attentional functioning, or that the arousing properties of CRH, which may be necessary for attentional functioning, might be taken over by other ligands of the CRH family such as UCN, UCNII or UCNIII.

The role of CRHR1 and CRHR2 in sustained and divided attention

The effects of lack of CRHR1 and CRHR2, and acute blockade of CRHR1 were investigated in subsequent experiments. Animals lacking CRHR1 learned the task equally well and as fast as wildtype animals. CRHR2 knockouts also learned to the task as well as wildtype mice, but at a lower rate. However, CRHR2 knockout mice did not display altered performance in parameters that are thought to reflect attention. Instead, CRHR2 knockout mice made significantly less errors of omission when measured over several sessions or under conditions of increased attentional load. This is not likely to be due to altered attentional processes, but may be due to

increased motivation. Spina and co-workers (1996) showed that i.c.v. administration of UCN decreased food intake in food deprived animals as well as in free-feeding rats. In fact, CRHR2 is expressed in the ventromedial hypothalamus (Chalmers et al., 1995), which makes this CRH receptor subtype a likely candidate for mediating the food intake suppressive effects of UCN. Mice lacking CRHR2 have been reported to have normal basal feeding and weight gain, whereas food intake is decreased following food deprivation (Bale et al., 2000; Kishimoto et al., 2000). However, UCN inhibited food intake for a longer period in wildtype animals than in CRHR2 knockouts, indicating that CRHR2 is involved in the suppressive effects of UCN on food intake (Conti et al., 1994). This suggests that the reduced number of errors of omission could be due to altered motivational drive.

CRHR1 knockout, in contrast, show reduced accurate responding. However, this difference in accuracy, albeit significant, was marginal (56.72 % vs. 50.07 %). The fact that groups only started to dissociate after a few sessions also argues against a pure attentional deficit and opens the possibility that other factors contributed to these results. Indeed, learning deficits have been reported in mice lacking CRH1 (Contarino et al., 1999). However, such a learning deficit would have to be regarded as subtle as animals perfectly well acquired the initial discrimination in the present set of experiments, and the deficit only became apparent under conditions of high task difficulty.

This accuracy deficit in CRHR1 ko mice was also seen under conditions of short ITI durations, i.e., when stimuli were presented at faster rate than expected, but it was independent on the length of the ITI, which would also argue against a pure

attentional deficit. An increase of ITI duration seemed to have even beneficial effects in that groups did no longer differ during these latter sessions with the exception of an increase in incorrect response latencies in mutant mice.

Acute blockade of CRHR1 does not affect sustained and divided attention

Along similar lines, administration of the CRHR1 antagonist R121919 reduced responsivity, as indicated by increased omissions and decreased reinforcers earned. However, R121919 does not alter performance in terms of parameters reflecting attention. Moreover, R121919 also failed to improve performance in CRH overproducing animals, suggesting that the deficit seen in the overexpressors was not mediated through overactivation of this receptor subtype. In contrast, subchronic administration of R121919 has been reported to attenuate the increased anxiety-related behaviour seen in CRH overexpressing mice (Min et al., 2000), again indicating a dissociation between the effects of CRH excess on anxiety- and stress-related behaviour, which may be mediated via the CRHR1 or, and the effects of CRH overactivity on attention and/or arousal. One possibility could be that the effects of CRH on this type of behaviour are mediated via the CRHR2, although lack of CRHR2, in absence over excessive CRH, does result in alterations in visuospatial attention. Alternatively, repeated administration of R121919 may be necessary to restore alterations secondary to live-long CRHR1 overstimulation.

Effects of cholinergic drugs are altered in mice with mutations in the CRH system

Interestingly, manipulation of the cholinergic system affected both CRH overexpressors and wildtype animals to different degree. Although the effects of

scopolamine on accuracy were comparable between groups, the effects of scopolamine on the number of reinforcers earned were more pronounced in wildtype mice. Likewise, physostigmine had stronger effects in wildtype than in transgenic animals at high doses (0.5 mg/kg), suggesting again decreased sensitivity of CRH overexpressors to cholinergic manipulation, i.e., to the response depressing effects of the drug. The errors of omission and the number of reinforcers measures confirmed this suggestion. Strikingly, scopolamine was also more potent in disrupting performance in wildtype animals compared to CRH knockout mice. This suggests that cholinergic functioning is altered following CRH hypo- as well as hyperactivity. However, differences in pharmacodynamics and/or pharmacokinetics in mutant animals cannot be ruled out.

One possibility could be that CRHR1 blockade led to further decrease in cholinergic activity, thereby leading to interaction with scopolamine. However, this effect is unlikely to be mediated via the nucleus basalis magnocellularis-cortical axis as hardly any co-localisation between CRHR1- and choline-acetyltransferase immunoreactivity has been observed at this level (Sauvage and Steckler, 2001). Lesions of the nucleus basalis magnocellularis lead to reduced accuracy in the 5-CSRTT (Muir et al., 1992; Muir et al., 1994; Robbins et al., 1989), and the lack of CRHR1 at this level could explain the unaltered attentional function in 5-CSRTT in CRHR1 and CRH knockout animals. Indeed R121919 failed to affect accuracy, alone or in combination with scopolamine. Although CRHR1 receptors are highly co-localised at cholinergic neurons in the medial septum and the diagonal band of Broca (Sauvage and Steckler al., 2001), these areas are unlikely to be involved in the modulation of attentional function in the 5-CSRTT. Lesions of the vertical limb of

the diagonal band, from which cholinergic projections to the cingulate cortex originate, do not alter 5-CSRTT performance in rats (Muir et al., 1996; Muir et al., 1992), and lesions of the hippocampus, i.e., of the target area for cholinergic projections arising from the medial septum, have been reported to also have no effects on a seven-choice serial reaction time task (Burk et al., 1999). Of course, it remains a possibility that the interaction between CRHR1 blockade and muscarinic blockade takes place at brainstem level, involving the pedunculopontine tegmental nucleus or laterodorsal tegmental nucleus projections to the thalamus, or at cortical or other subcortical levels known to be involved in the mediation of performance on that task, such as the medial prefrontal cortex, as lesions of this cortical area, i.e., of a brain area rich in CRH-containing neurons and CRHR1 (Chalmers et al., 1995; Wilcoxon et al., 2000; Swanson et al., 1983), have been reported to also impair accuracy and response latencies (Muir et al., 1996).

Alternatively, it is possible that CRHR1 blockade interfered with dopaminergic neurotransmission as dopaminergic neurons in both the substantia nigra pars compacta and the ventral tegmental area strongly express CRHR1 (Sauvage et al., 2001). Moreover, dopaminergic neurotransmission is stimulated by CRH (Lavicky and Dunn, 1993), and lesions of the mesolimbic dopaminergic system in rats slow responding without affecting accuracy in the 5-CSRTT (Cole and Robbins, 1989), i.e., induce effects comparable to those seen following R121919 administration.

To summarise, visuospatial attention is not affected by lack of CRH or CRHR2. Functional deletion of CRHR1, however, results into impaired performance in the 5-CSRTT, although this deficit developed over sessions, which argues against a pure

attentional deficit. Excessive CRH activity disturbs attentional functioning, which cannot be reversed by acute blockade with a CRHR1 antagonist. However, CRH overproduction also results alteration in various neurotransmitter systems, and therefore chronic inhibition of CRH transmission via CRHR1 might be necessary to restore visuospatial attention. Alternatively, it is possibly that disruption of attentional processes occurs via CRHR2. This question can be tackled by investigating effects of novel compounds blocking CRHR2.

Conclusions

From the results reported in this thesis, the following points can be concluded: chronic CRH excess increases some, but not all, aspects of anxiety. More specifically, innate anxiety seems to be disrupted, while conflict and fear may be spared. These effects were reversed by long-term treatment with citalopram. Interestingly, citalopram has beneficial effects in both patients suffering from anxiety disorders as well as depressive patients. Moreover, 5-HT_{1A} receptor challenge failed to activate the HPA axis in CRH overexpressing mice, while the hypothermic response following 5-HT_{1A} stimulation remained intact, which bears further resemblance to the situation seen in depressed patients. These findings suggest that CRH overexpressing mice may be a useful model of depression. More importantly even, these findings add to the growing literature implicating CRH into the pathophysiology of depression and anxiety disorders.

These conclusions received further support from findings on the attentional function of these animals. Overproduction of CRH induced an impairment in visuospatial attention and altered responsiveness to cholinergic drugs was seen in these mice.

These findings might have important implications for the development of novel strategies to treat depression and suggests that treatments aiming at normalizing the activity of the CRH system might be beneficial for a range of symptoms seen in this devastating disorder, including anxiety and cognitive dysfunction.

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List of abbreviations

5-CSRTT	5-choice serial reaction time task
5-HT	5-hydroxytryptamine
8-OH DPAT	8-hydroxy-2-(di-n-propylamino)tertalin
ACTH	adrenocorticotrophic hormone
AVP	arginine vasopressin
BNST	bed nucleus of the stria terminalis
CRH	corticotropin-releasing hormone
CRHR1	corticotropin-releasing hormone receptor 1
CRHR2	corticotropin-releasing hormone receptor
CSF	cerebrospinal fluid
DST	dexamethasone suppression test
ECT	electroconvulsive shock therapy
GR	glucocorticoid receptors
HPA	hypothalamus-pituitary-adrenocortical
i.c.v.	intracereboventricular
ITI	Inter Trail Interval
MR	mineralocorticoid receptors
ODNs	oligodeoxynucleotides
PVN	paraventricular nucleus
UCN	urocortin
UCNII	urocortin II
UCNIII	urocortin III

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Marcel M. van Gaalen, Thomas Steckler. Behavioural analysis of four mouse strains in an anxiety test battery. Behavioural Phenotyping Mouse Mutants Cologne Abstract Book, p.93 (2000).

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